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Efficiency of Standardized ILEAL Digestible Lysine Utilization for Whole Body Protein Deposition in Pregnant Gilts and Sows during Early, Mid and Late Gestation

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EFFICIENCY OF STANDARDIZED ILEAL DIGESTIBLE LYSINE UTILIZATION FOR WHOLE BODY PROTEIN DEPOSITION IN PREGNANT GILTS AND SOWS DURING EARLY, MID AND LATE GESTATION

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

AA	amino acid(s)
ANOVA	analysis of variance
BW	body weight
$BW^{0.75}$	metabolic body weight
°C	degree centigrade
Ca	calcium
CP	crude protein
Cys	Cysteine
d	day(s)
EBW	empty body weight
Eq.	equation(s)
exp	natural logarithm
g	gram
GIT	gastrointestinal tract
H_0	null hypothesis
IAAO	indicator amino acid oxidation
kg	kilogram

kcal	kilocalories
kSID Lys WB Pd	efficiency of SID Lys utilization for whole body protein deposition
Leu	leucine
LS	least square
Lys	lysine
m	meter
maint	maintenance
MCP	monocalcium phosphate
ME	metabolizable energy
Met	methionine
mg	milligram
mL	milliliters
mm	millimeter
N	nitrogen
NRC	National Research Council
P	phosphorus
Pd	protein deposition
P1	first parity

P2	second parity
reqt	requirement
SAS	Statistical Analysis System
SEM	standard error of the mean
SID	standardized ileal digestible
t	time
Thr	threonine
TNF-α	tumor necrosis factor alpha
Trp	tryptophan
Val	valine

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ABSTRACT

EFFICIENCY OF STANDARDIZED ILEAL DIGESTIBLE LYSINE UTILIZATION FOR WHOLE BODY PROTEIN DEPOSITION IN PREGNANT GILTS AND SOWS DURING EARLY, MID AND LATE GESTATION

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The total daily amino acid (AA) requirements for pregnant sows are based of Lys which is the first-limiting AA in swine diets. The SID Lys requirements are estimated by the NRC (2012) gestating sow model as the sum of those required for maintenance and protein retention divided by the efficiency of SID Lys utilization. The NRC (2012) gestating sow model assumed AA efficiency is constant across stages of gestation and parities; however, this does not reflect the change in metabolic demand of various age sows at different stages of gestation. Therefore, an experiment was conducted to evaluate the efficiency of SID Lys utilization for whole body protein deposition (kSID Lys WB Pd) in pregnant sows during early, mid and late gestation. Three 12 d N-balance studies were conducted to represent different periods of gestation for gilts, P1 and P2 sows in two blocks. Graded levels of SID Lys below the NRC (2012) requirements were used to estimate the kSID Lys WB Pd within balance periods. The kSID Lys WB Pd was defined as the slope of the linear relationship between SID Lys intake and SID Lys retention. The kSID Lys WB Pd for gilts was 0.71, 0.37 and 0.52 for early, mid and late gestation, respectively; for P1 sows the kSID Lys WB Pd was 0.56, 0.38 and 0.48 for early, mid and

late gestation, respectively; for P2 sows the kSID Lys WB PD was 0.44 and 0.65 for early and late gestation, respectively. The kSID Lys WB Pd could not be determined for mid gestation in P2 sows due to data failure to meet statistical assumptions (i.e. normality of residuals). The kSID Lys WB Pd was greatest in early gestation for gilts and late gestation for P2 sows and lowest for all parities in mid gestation. Our present study is important to refine the AA requirement models for gestating sows and suggest the need for parity-segregated phase feeding.

CHAPTER 1

Lysine requirements for pregnant sows: literature review

1.1. Introduction

The efficient use of dietary protein by pigs depends on the AA supply in relation to the animal's requirements. With the increasing availability of crystalline AA, diets with the desired quantity and relationship of AA can be formulated. There is a growing interest in formulating low crude protein diets with a well-balanced AA content that provide for optimal animal performance and limit excretion of excess nutrients (i.e. precision feeding). This strategy requires good knowledge of AA requirements throughout pig's life. However, the information regarding requirements of pregnant sows is still limited (NRC, 2012). The lack of accurate requirements limits the application of precision feeding of sows. In addition, amino N consumed in excess is excreted as urea, which contributes to the environmental impact of animal production and increases costs. On the other hand, deficient AA supply can decrease performance and productivity.

Using the ideal protein concept, the requirements of AA are expressed relative to the requirement for Lys because it is typically the first-limiting AA in pig diets. This approach has practical implications because nutritionists only need to know the Lys requirement and, in combination with a constant ideal protein profile, the ratio between the AA (van Milgen and Dourmad, 2015). Because of this, more research has been carried out on the Lys requirements, than on any other AA; however, there is very little known about the dynamics of Lys requirements during pregnancy. In fact, due to a limitation in available empirical data, the NRC 2012 gestating sow model manipulated

the kSID Lys WB Pd in order to be consistent with the only three Lys requirement studies available (NRC, 2012).

1.2. Empirical estimates of AA requirements for gestating sows

The information available on AA requirements in gestating sows is limited compared to that of growing pigs and lactating sows, which gives us an idea of the difficulty of its estimation. The performance criteria in growing pigs and lactating sows (i.e. growth rate and carcass quality and milk composition, respectively) are easily measured whereas protein deposited in pregnancy-related tissue is less easily measured (Sohail et al., 1978). In an effort to advance knowledge in this area, techniques of short duration, but with an adequate degree of reliability have been developed. Indicator AA oxidation (IAAO) and N balance methods are considered the more reliable for estimating AA requirements in gestating sows, but the comparative slaughter technique, plasma urea N and plasma AA concentrations, and sow body weight measurements have also been used (Dourmad and Etienne, 2002; Bérard and Bee, 2010; Levesque et al., 2011; NRC, 2012). Depending on the method used, the determined requirements may vary. For example, greater requirement estimates have been determined when using plasma urea N concentrations compared to the slaughter technique (Dourmad and Etienne, 2002) and the use of plasma AA concentrations has been demonstrated to be poorly correlated with other requirement estimate techniques (Levesque et al., 2011). The IAAO method has been suggested as the “gold standard” method for evaluating AA requirements (Mok, 2015). This is followed by the N balance method despite the fact that numerous studies report that N balance overestimates the requirements (Just et al., 1982; Quiniou et al.,

1995; Lenis et al., 1999; De Lange et al., 2001). However, no direct comparison between IAAO and N balance has been conducted. All these methods are widely used and have proven useful in detecting differences between dietary treatments (Zervas and Zijlstra, 2002), but special attention must be paid when used for the prediction of N status, protein deposition (Pd) and requirements of AA in pigs.

Nutrient requirements have traditionally been based solely on a summary of empirical studies using the previously mentioned methods. However, there are limitations in this approach; most of the research carried out in AA requirement estimation did not consider or report the same variables nor use the same method of statistical analysis, so comparison and extrapolation of the data is limited. As a consequence, more emphasis has been placed on the factorial estimation of AA requirements (NRC, 2012). The factorial approach was the basis for defining the InraPorc and NRC model requirements (van Milgen and Dourmad, 2015); both models are similar because they are adaptations of the Dourmad et al. (2008) gestating sow model. In fact, when parameterizing both models with similar conditions there is only a 3% difference in the predicted SID Lys requirement during pregnancy, with the InraPorc model being greater (van Milgen and Dourmad, 2015). Because of this, only the NRC 2012 model will be described and analyzed for the rest of this literature review.

1.3. NRC 2012 Gestating Sow Model

The NRC (1998) introduced the use of computer models to estimate AA requirements and developed three models: growing-finishing pigs, gestating sows, and lactating sows. The NRC (2012) improved these models by converting them into

mechanistic, dynamic and deterministic models better representing nutrient and energy use in the whole animal (NRC, 2012).

The NRC (2012) gestating sow model is an improvement over the previous model and includes more than 30 years of research on energy and AA utilization by the pregnant sow and it is an important decision support tool for understanding sow nutrition and the associated performance. However, there were clear limitations in its development (NRC, 2012). The current research focuses on the efficiency of Lys utilization and it is essential to understand how the model works in order to have a clearer understanding of its meaning in estimating AA requirements.

1.4. Estimation of SID Lys requirements by the NRC 2012 gestating sow model

The requirements of AA are expressed relative to the requirement of Lys because Lys is the first-limiting AA in pig diets. Using the ideal protein concept (i.e. knowing the Lys requirements in combination with an ideal protein profile), the other essential AA requirements can be estimated. The NRC 2012 gestating sow model follows 5 steps in the estimation of SID Lys requirements for pregnant sows and are described below.

1.4.1. Estimation of body protein (Step 1).

The estimation of the maternal body protein is a function of the empty body weight (EBW) and backfat (in mm) measured at 6-8 cm away from body midline at the last rib level. The EBW is assumed to be 4% of the maternal body weight (BW). Thus, the maternal body protein can be estimated with the following equations:

$$Maternal\ EBW(kg) = 0.96 \times maternal\ BW \qquad (Eq. 8-49;\ NRC, 2012)$$

$$Maternal\ body\ protein(kg) = 2.28 + 0.178 \times maternal\ EBW - 0.333 \times backfat \qquad (Eq. 8-51;\ NRC, 2012)$$

Backfat thickness and live weight have been reported to be accurate in estimating body composition only in animals that are physiologically similar to the population in which the equations were derived (Rozeboom et al., 1994; Landgraf et al., 2006). In this sense, Eq. 8-51 was developed from one study using 23 Large White gilts, of which 7 animals were sacrificed at farrowing and 16 animals at weaning (Dourmad et al., 1997); Thus, these equation may not represent the body composition throughout gestation nor the body composition of all genetic sires.

1.4.2. **Estimation of growth of conceptus and protein pools (Step 2).**

In this second step the protein content (kg) in the body of the sow is divided into six different protein pools, identified as: fetus, placenta plus fluids, uterus, mammary tissue, time-dependent maternal Pd, and energy intake-dependent maternal Pd. Maternal body protein content predictions are based in the latter two pools of protein previously mentioned. The equations that predict the weight of the conceptus (fetus and placenta plus fluids), uterus, and mammary tissue are from Dourmad et al. (1998) where the authors combined equations developed by (Noblet et al., 1985).

Pool 1: Fetus protein deposition

The equation that predicts the fetus weight (Eq. 8-56) uses natural logarithmic value and is a function of time and litter size and allow estimations at any given day of gestation.

$$Fetus\ Pd(g) =$$

$$exp\{8.729\ -\ 12.5435\ \times\ exp(-0.0145 \times t)$$

$$+ 0.0867\ \times\ litter\ size\} \qquad (Eq. 8-56;\ NRC, 2012)$$

Equation 8-56, however, was developed 30 years ago from a population of 26 Large White gilts, with a litter size ranging from 9 to 14 (Thomas et al., 2018). Nowadays, total born averages more than 14 pigs in some of the most prolific sow herds. The estimations are unrealistically high when applying this equation to sows with more than 14 pigs born alive. Because of this, the protein deposited in the fetus is corrected for mean piglet birth weight according to the equation 8-58.

$$Ratio = (litter\ size \times average\ piglet\ birth\ weight, g)/$$

$$1.12\ \times\ exp\ \{[9.095\ -\ 17.69\ exp\ (-0.0305\ \times\ 114)$$

$$+\ 0.0878\ \times\ litter\ size]\} \qquad (Eq. 8-58;\ NRC, 2012)$$

The numerator of the ratio is the actual litter birth weight and the denominator is derived from Dourmad et al. (1999). It is not known what the value 1.12 represents and details are not reported in the NRC. The visual representation of Eq. 8-56 corrected according Eq. 8-58 is shown in Fig. 1-1A.

Pool 2: Placenta plus fluids protein deposition

The protein content in placenta and fluids is represented as a function of time and anticipated litter size, using a Michaelis–Menten kinetics function. This equation is also corrected for actual litter birth weight according to Eq. 8-58 (NRC, 2012). The visual representation of Eq. 8-57 corrected according Eq. 8-58 is shown in Fig. 1-1B.

$$Placenta\ plus\ fluids\ Pd(g) =$$

$$\left[38.54 \times \left(\frac{t}{54.969}\right)^{7.5036}\right] / \left[1 + \left(\frac{t}{54.969}\right)^{7.5036}\right] \qquad (Eq. 8-57;\ NRC, 2012)$$

Pool 3 & 4: Empty uterus and mammary tissue protein deposition

The protein content of the empty uterus and mammary gland are estimated based on natural logarithmic values as a function of time (Eq. 8-59 and 8-60). The visual representation of Eq. 8-59 and Eq.8-60 is shown in Fig. 1-2.

$$Uterus\ Pd\ (g) =$$

$$exp\ [6.6361 - 2.4132 \times exp\ (-0.0101 \times t)] \qquad (Eq. 8-59;\ NRC, 2012)$$

$$Mammary\ tissue\ Pd\ (g) =$$

$$exp\ \{8.4827 - 7.1786 \times exp\ [-0.0153 \times (t - 29.18)]\} \qquad (Eq. 8-60;\ NRC, 2012)$$

Pool 5 & 6: Maternal Pd

Maternal body protein is made up of time-dependent maternal Pd and energy intake-dependent maternal Pd. Protein retention in the time-dependent and energy-intake dependent maternal body protein pools was estimated from whole-body N retention at different stages of gestation according to Dourmad et al. (1998). Time-dependent maternal body protein gain represents residual protein retention that could not be associated with energy intake or reproductive tissues. As protein gain in this pool occurs

only during the first part of gestation, after day 56 of gestation a protein gain value of 0 is forced and protein gain is predicted using a Michaelis–Menten kinetics function (Eq. 8-61). NRC 2012 considered the values obtained with Eq. 8-61 elevated, thus, the relative values of 0.84, 0.75, 1.00, and 1.36 were used as adjustment factors in the software but they are not part of the equation nor was it reported how they were implemented (NRC, 2012). The visual representation of Eq. 8-61 is shown in Fig. 1-3A.

$$Time - dependent\ maternal\ Pd(g) = [(1522.48) \times (56 - t)/36]^{2.2}/ [1 + (56 - t)/36)^{2.2}] \qquad (Eq. 8 - 61;\ NRC, 2012)$$

Daily energy-intake dependent maternal Pd is linearly related to metabolizable energy (ME) intake above maintenance ME requirements on day 1 of gestation (Eq. 8-62). In order to achieve a reasonable fit between observed and estimated changes in the sow's body composition across parities, the coefficient 'a' was added to the equation (Eq. 8-63). The model user can add an adjustment to match observed with predicted sow BW changes and changes in the backfat thickness (NRC, 2012).

$$Energy - intake\ dependent\ Pd\ (g/day) = a \times (ME\ intake\ -\ maintenance\ ME\ requirements\ on\ day\ 1\ of\ gestation, kcal/day) \times adjustment \qquad (Eq. 8 - 62;\ NRC, 2012)$$

$$Coefficient\ a = (2.75\ -\ 0.5 \times parity) \times adjustment\ ;\ a > 0 \qquad (Eq. 8 - 63;\ NRC, 2012)$$

The visual representation of Eq. 8-62 with coefficient ‘a’ estimated from Eq. 8-63 without adjustment is shown in Fig. 1-3B. The summary of the estimation of the various protein pools according to NRC (2012) gestating sow model is shown in Fig. 1-4.

1.4.3. **Lysine content in each protein pool and endogenous losses (Step 3).**

The Lys content of the maternal body protein was taken from Everts and Dekker (1995), which was determined on 14 gilts (Large White X Dutch Landrace) at day 108 of gestation and excluded uterus, fetus, and hair, but included the mammary gland. The Lys content of the fetus was estimated from the study of Wu et al. (1999) and mammary gland Lys content was estimated from Ji et al. (2006) with samples analyzed by Evonik-Degussa according to Llames (1994). For the fetus and mammary gland, the concentrations of Lys (g/100 g CP) were linearly regressed against the respective day of gestation, with a forced intercept of 0. The reported values in Table 1-1 are the slopes of each linear regression. Lysine concentration for placenta and fluids were determined similarly as for the mammary gland from a total of 22 gilts on d 43, 57-58, 90, 92, and 100-109 of gestation. The Lys content in placenta and fluids was averaged to represent one Lys content on day 45 of gestation (NRC, 2012). The uterine AA profile was obtained from the same gilts as described for placenta fluids plus eight additional nonpregnant gilts; similarly, as Lys in placenta and fluids, the Lys content in the uterus was averaged. Thus, the Lys content relative to protein, in each protein pool is assumed to be constant by parity and stage of gestation.

Table 1-1. Lysine content in each of the 6 protein pools considered by the NRC (2012) gestating sow model.

Maternal body (time-dependent & energy dependent Pd)	**Fetus**	**Uterus**	**Placenta & Fluid**	**Mammary gland**
Lysine, g/100 g CP				
6.74	4.99	6.92	6.39	6.55

However, this approach has its limitations. The estimates are made as a function of the weight of each protein pool and not its composition. For example, maternal body protein is estimated as a function of BW; however, it is possible that some body fat can be metabolized and replaced with water in the maternal body (Moe et al., 1971). As water has a higher density than fat, this transient replacement of fat by water can increase BW but not the maternal body protein. Moreover, Wu et al. (1999) reports that the AA composition of the fetus varies across gestation, as shown in Fig. 1-5.

1.4.4. **Basal endogenous losses (Step 4).**

In addition to the 6 protein groups previously described, the NRC (2012) gestating sow model includes integument and basal endogenous GIT losses as tissues that demand AA. Basal endogenous GIT losses are related to feed intake and are calculated with Eq. 8-40. Integument losses are a function of metabolic weight ($BW^{0.75}$) as shown in Eq. 8-41. These equations do not discriminate by parity or stage of pregnancy. This

approach is similar to those for growing-finishing pigs, except that the GIT Lys losses are assumed to be 0.5053 g of Lys per kg of feed intake (NRC, 2012).

$$Basal\ endogenous\ GIT\ lysine\ losses(g/day) = feed\ intake \times 0.5053 \quad (Adjustment\ from\ Eq. 8-40;\ NRC, 2012)$$

$$Integument\ lysine\ losses\ (g/day) = 0.0045\ \times\ BW^{0.75} \quad (Eq. 8-41;\ NRC, 2012)$$

Finally, total Lys retention is calculated by adding the Lys content in each protein pool, integument losses and endogenous losses.

1.4.5. **Efficiency of SID Lys utilization for whole body protein deposition (Step 5).**

According to NRC (2012), the kSID Lys WB Pd was estimated from three of the four following Lys requirement studies, all of them using the N balance method. NRC (2012) does not specify which three studies were selected: Rippel et al. (1965) conducted studies between day 100 and 110 of gestation, Duée et al. (1975) initiated studies on day 80 of gestation, and Woerman and Speer (1976) initiated their studies on days 0, 30, 60, and 95 of gestation. Étienne and Dourmad (2002) conducted four N balance study periods between day 20 and 104. Those results were contrasted with Everts and Dekker (1995) who estimated a Lys efficiency of 0.46 at an average daily N intake of 74.4 g and 0.59 at an average daily N intake of 50.8 g. Based on the studies of [insert], the kSID Lys WB Pd was estimated to be 0.49. This is 65.3% of the maximum efficiency (equivalent to the efficiency of Lys utilization for maintenance: 0.75). Additionally, because there are

currently no direct estimates, the efficiency of AA utilization for protein retention for all AA was assumed to be 65.3% of the efficiency of maintenance with minor adjustments (NRC, 2012).

For model development, NRC (2012) assumed that the efficiency of Lys utilization for whole body Pd is identical across parity, days of gestation, the various protein pools, and endogenous and integument losses.

The SID Lys requirements are estimated, according to Eq. 8-66. For a better understanding, Eq. 8-66, can be rewritten as Eq. 8-66a and Eq. 8-66b. In Eq. 8-66b, it can be noted that the SID Lys requirements are calculated by dividing the Total Lys retention by the kSID Lys WB Pd. The value of 62.9% was used instead of the 65.3%, reported above, because an adjustment that accounts for between-animal variability was made (NRC, 2012).

$$SID\ Lys\ requirements\ for\ SID\ Lys\ retention\ (g/day) = \frac{Total\ lysine\ retention}{0.75} \times 1.589 \qquad (Eq. 8-66;\ NRC, 2012)$$

$$SID\ Lys\ requirements\ for\ SID\ Lys\ retention\ (g/day) = \frac{Total\ lysine\ retention}{0.75 \times \frac{1}{1.589}} \qquad (Eq. 8-66a;\ Adjustment\ from\ Eq. 8-66;\ NRC, 2012)$$

$$SID\ Lys\ requirements\ for\ SID\ Lys\ retention\ (g/day) = \frac{Total\ lysine\ retention}{0.75 \times 0.629} \qquad (Eq. 8-66b; Adjustment\ from\ Eq. 8-66;\ NRC, 2012)$$

1.4.6. **Manipulation of the Efficiency of SID Lys utilization for whole body protein deposition.**

After all the calculations previously shown, the model includes a manipulation. As the NRC (2012) states: “The gestating sow model was forced to be consistent with three carefully selected Lys requirement studies, by manipulating the efficiency of using SID Lys intake for Lys retention in Pd …”. The NRC (2012) also reports that the efficiency of SID Lys utilization for whole body protein deposition was forced to match model-generated requirements to requirements presented in NRC (1998) for gestating sows. This can be observed in the Fig. 1-6. The exact coefficients by which the model was manipulated, or the kSID Lys WB Pd that was used are not shown in the NRC (2012) gestating sow model documentation.

Therefore, the estimation of AA requirements according to the NRC (2012) gestating sow model is only congruent with three N balance studies. Navales et al. (2018) speculated that the AA requirements established by the NRC (2012) gestating sow model are overestimated in the first two thirds of the gestation and underestimated in the last third. One possible explanation to for these inaccurate estimations is due to the method used to determine the AA requirements, as will be discussed in the next section.

1.5. Nitrogen Balance

Nitrogen retention has been shown to be overestimated when determined by N balance and the difference is important (up to 16%) and variable when compared with the comparative slaughter technique (Just et al., 1982; Quiniou et al., 1995; Lenis et al., 1999; De Lange et al., 2001). This difference may be due to the overestimation of intake

and underestimation of excretion. Pregnant sows are restrictively fed, thus overestimation of intake is less of an issue, but underestimation of N in feces and urine from incomplete collection (e.g. loss of gaseous ammonia in urine) is still an important factor to consider. Lenis et al. (1999) reported that when feeding growing pigs at a N intake ranging from 25 to 40 g per animal per day and collecting urine in sulfuric acid (40% wt/vol), N loss from volatilization was in between 1 to 2%. Additionally, Just et al. (1982) reported that in respiration experiments, ammonia collected from urine and feces amounted to 0.5-1.5% of the N intake, what caused a 2-5% overestimation of the N balance. Quiniou et al. (1995) reported an overestimation of the N retention from 2.9% to 3.9% due to gaseous N losses.

It has been also reported that freeze drying fecal samples can result in N losses up to 2% and it is recommended to perform N analysis on undried feces (Just et al., 1982; Quiniou et al., 1995). Other sources of error may occur throughout the process, such as feed waste or errors when taking representative and homogeneous samples, which may seem simple, but in practice it may be difficult (Just et al., 1982). Especially large volumes of urine output in a 24-h period, for example, the urine produced by gilts, can reach about 50 kg per day (Miller et al., 2018).

In an experiment that studied the variability of daily urinary N excretion of gestating gilts using Foley catheters for total urine collection, Miller et al. (2018) reported that only 67% of the daily urine collections were successful (not considering the unsuccessful catheterization), 25.5% of the observations were partial collections, and 7.5% was lost information because of the disconnection of catheters. In addition, 12% of the observations were outliers. Miller et al. (2018) considered urine N values as outliers if

they contributed to a coefficient of variation greater than 20%, since a more rigorous outlier selection may skew the observations towards sows that have a lower or more consistent water intake. It is important to mention that higher the coefficient of variation, the greater the level of dispersion around the mean and less precise the estimate.

1.5.1. **Open-catheter system.**

The use of Foley catheters is the standard method to accomplish total urine collection during N balance studies using pregnant sows (Van den Brand et al., 2000; Dourmad and Etienne, 2002; Srichana, 2006; Miller et al., 2016). Foley catheters are attached to collecting tubes that drain into buckets next to the stall, the so-called "open catheter system". A similar system was used in humans in the first half of the 20th century but replaced in the 1960s by "closed" catheter systems, since bacteriuria occurred on the fourth day (Warren, 2001). In closed catheter systems plastic bags are fused to the distal end of the tubes which protect the lumen from the contaminated environment. With this system in humans, the onset of bacteriuria is more than 30 days (Warren, 2001). It is not known when bacteriuria occurs in the sow, but because the catheters are not placed under aseptic conditions as in humans, and they are open systems, there is a high risk of bacteriuria during the 5 days of collection. Most of the bacterial strains that enter the catheterized urinary tract can multiply to high concentrations in a day and since catheter drainage is often imperfect, bacteria can survive in the volume of urine remaining in the bladder (Warren, 2001). Bacteriuria not only can jeopardize the animal's health, but also affects the estimation of N retention.

1.5.2. **Non-dietary sources of N in urine.**

Urine may contain non-dietary sources of N, which include cells and metabolites. Major cells found in urine may come from the blood, epithelial, or bacterial cells. Hematological origin cells are made up of cells such as erythrocytes, leukocytes, neutrophils, glitter cells, lymphocytes and eosinophils. In theory, any of the white blood cells could be present in the urine (Ringsrud, 2001). Additionally, cells can come from the kidney. Renal epithelial cells normally found in urine are caused by normal exfoliation, but their presence can be increased by fever, inflammation, and infection. Other types or epithelial cells including urothelial and squamous cells, although present in smaller amounts, can also be increased during infection (Ringsrud, 2001).

Urine is usually sterile, but infection can be caused by bacteria or yeast. During an infection, macrophages and phagocytes secrete a variety of cytokines, the exact role of these metabolites in the defense against infection is not yet known, but some of their functions include attracting more phagocytes to places where the pathogen has invaded the body and to mimic the action of antibodies (Janeway et al., 2005). The presence of these cytokines and microorganisms themselves during an infectious process could increase the N content in the urine, although these values could be negligible compared to the endogenous production of another group of metabolites: nitrites.

The main source of endogenous nitrate in mammals is the L-arginine-nitric oxide pathway. During systemic inflammatory reactions or infections, white blood cells and other cells express nitric oxide synthetases catalyzing the production of large amounts of nitric oxide from L-arginine (Lundberg et al., 2004). Nitric oxide reacts with hemoglobin and other compounds to form nitrate. It has been reported that nitrites are bactericidal for

several microorganisms, among them the most common uropathogens such as *E. coli*. There is clear evidence that the production of nitrates is an immune response. In fact, a urinary test-strip for nitrite is widely used to detect infections of the urinary tract (Lundberg et al., 2004). There are also reagent-strips for testing blood, proteins, and leukocyte esterase (Ringsrud, 2001).

One of the limitations of the N balance trials for determining protein deposition in pregnant sows is that it is only possible to know the total protein deposition (i.e. products of conception and maternal body). These protein pools can be measured separately using the comparative slaughter technique.

The application of the N balance method appears to be more reliable when response levels are not the main goals of the experiment, but the relative differences among treatments (Just et al., 1982). Even so, it is one of the most widely used methods for the determination of AA requirements (NRC, 2012) and it is important to emphasize that the validity of N balance studies depends on the experimental design, the accuracy of the chemical analysis, and a careful execution of each step of the experiment (Just et al., 1982).

1.6. Changes in kSID Lys WB Pd in gestating sows

The kSID Lys WB Pd of pregnant sows has been reported as a constant value of 0.49 independent of parity and stage of gestation (NRC, 2012); however, there is evidence to suggest that the kSID Lys WB Pd varies across stages of gestation and by parity. The kSID Lys WB Pd includes the efficiency of SID Lys utilization for maternal Pd and conceptus Pd. The use of the term “efficiency of utilization for conceptus development” has been used for more than 30 years in ruminant nutrition, although it has been limited

to reference to energy (Robinson et al., 1980; Haresign and Cole, 1983; Kiani, 2007). This distinction has not been made in swine, although the physiology of the tissues involved in conceptus development and research done in growing pigs suggest that the efficiency of SID Lys utilization for conceptus Pd is different than the efficiency of SID Lys for maternal Pd (Jansson et al., 1998; NRC, 2012; Zhang et al., 2018).

The efficiency of SID Lys utilization for conceptus development may be influenced by the different tissues that make it up and that begin to demand AA at different times throughout pregnancy. Fetal protein accretion rate increases after d 69 of gestation (Kim et al., 2005) and fetal efficiency of AA utilization is influenced by the activities of the placental transporters, placental AA metabolism, membrane potential, and available exchange area (Jansson et al., 1998). Blood flow also affects the efficiency of AA utilization because it is an important determinant of substrate concentration at the site of transport (Lewis et al., 2013). Maternal artery flow affects the rate at which arterial blood is supplied to the placenta and the rate at which AA depleted blood is removed. Fetal umbilical blood flow determines the rate at which umbilical arterial blood is delivered and the rate at which AA are removed from the exchange site across the placenta (Lewis et al., 2013). Maternal blood flow and uterine blood flow are closely related to fetal growth and litter size (Père and Etienne, 2000). Because the fetal protein accretion rate varies across gestation and the AA delivery through the placenta to the fetus is influenced by factors that vary throughout gestation (e.g. blood flow) the efficiency of AA utilization for fetal PD should also vary. Additionally, because multiparous sows tend to have heavier litters compared to gilts, the demand for AA should be greater and probably influences the efficiency of AA utilization for fetal Pd.

Placenta grows quickly between d 20 and 60 and has achieved maximum size by d 70 of gestation (Knight et al., 1977; Wu et al., 2005). During the formation of placenta and allantoic fluid, AA biosynthesis should occur; approximately 50% of the total free AA present in allantoic fluid is arginine and ornithine and it is believed that allantoic fluid is a reservoir of nutrients (Wu et al., 2010). Arginine and ornithine can be biosynthesized through the urea cycle using N scavenged from other AA like Lys. Thus, the efficiency of SID Lys utilization for conceptus development may be increased during placenta and allantoic fluid Pd due to biosynthesis of arginine and ornithine.

Mammary protein accretion mainly occurs after day 82 of gestation (Kim et al., 2005) and AA utilization is influenced by the activities of the AA transporters and maternal blood flow (Kim and Wu, 2009; Zhang et al., 2018). Litter size also influences mammary gland growth and SID Lys needs (Kim et al., 1999), which may affect the efficiency of SID Lys utilization for mammary gland Pd. As multiparous sows tend to have heavier litters compared to gilts the efficiency of SID Lys for mammary gland Pd may vary by parity.

There is evidence that shows that the efficiency of SID Lys utilization for maternal Pd also varies over time. Experiments with growing-finishing pigs revealed the marginal efficiency of using SID Lys for body Pd decline with increasing BW (NRC, 2012). Thus, the efficiency of SID Lys utilization for maternal Pd should to be greater for gilts compared to multiparous sows.

Additionally, the ratio between maternal Pd and conceptus Pd vary across gestation and parity. Reynolds et al. (1985) reported that in sows, insulin resistance increases as pregnancy progresses. Insulin resistance increases the AA concentration in

the maternal blood possibly for activating the accumulative transporters in the placenta (Broer, 2008) and mammary gland (Zhang et al., 2018) needed for transport of AA against concentration gradient. Increasing insulin resistance would therefore reduce maternal Pd and boost conceptus Pd as gestation progresses. Furthermore, there are reports that suggest that there are differences in insulin resistance by parity. Tumor necrosis factor alpha (TNF-α) has been used as a predictor of insulin resistance both in animals and humans (Barbour et al., 2007). Ison et al. (2018) reported that primiparous sows have lower plasma concentrations of TNF-α than multiparous sows, which would indicate that gilts are less insulin resistant compared to older sows.

Evidence presented thus far suggest that the efficiency of SID Lys utilization between maternal Pd and conceptus Pd differs and varies by parity and throughout gestation as well as the ratio between them. As the kSID Lys WB Pd depends on the efficiency of SID Lys utilization for maternal body Pd and conceptus Pd, the hypothesis of the present study is that the kSID LysWB Pd varies across stages of gestation and sow parity. The objective was to determine the kSID Lys as influenced by age and stage of pregnancy.

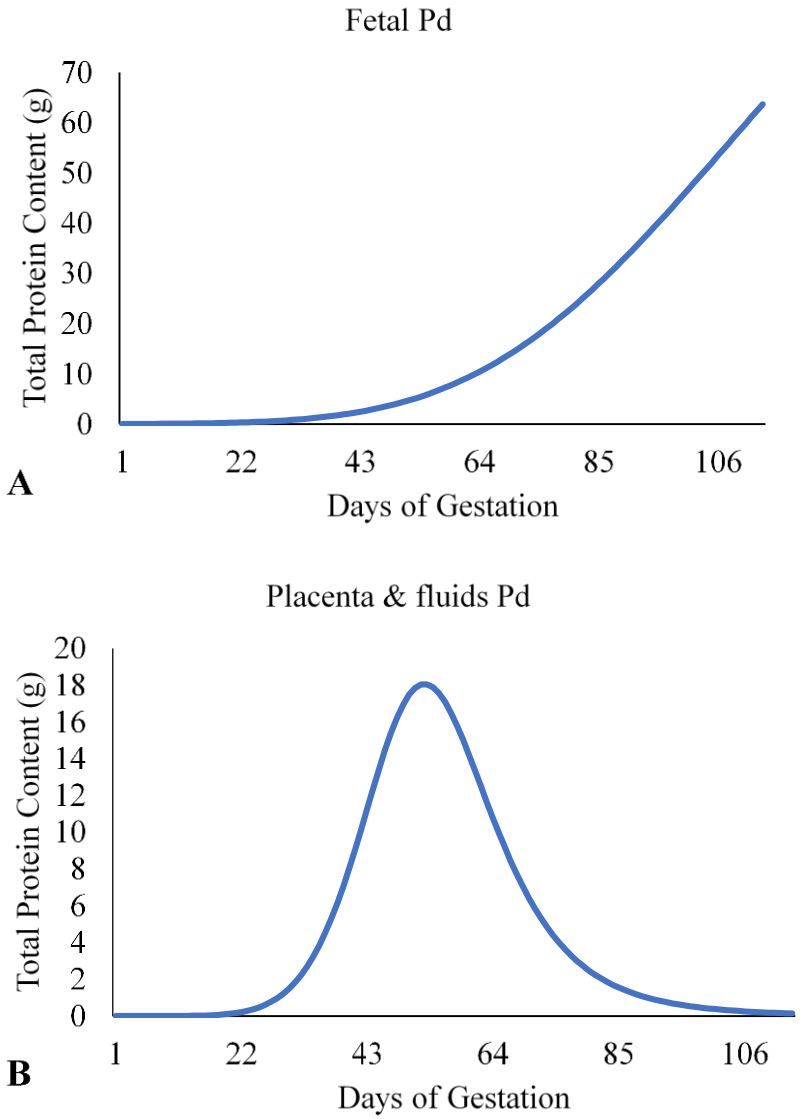

Figure 1-1. Fetal Pd (A) and placenta and fluids Pd (B) estimation by the NRC (2012) gestating sow model. The model was parameterized to represent a gilt weighing 140 kg at breeding (12.5 pigs/litter, 1.4 kg birth weight) fed 2.21 kg/d between breed to d 89 and 2.61 kg/d from d 90 to farrowing.

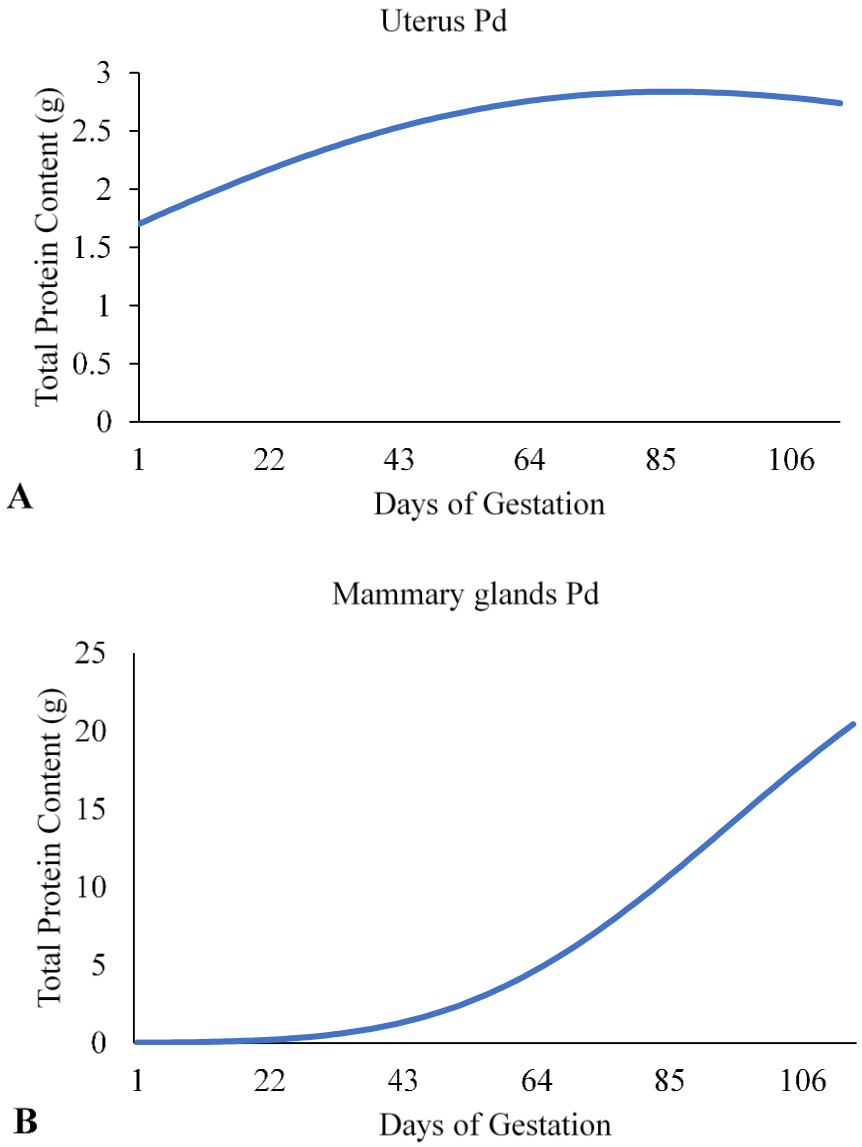

Figure 1-2. Uterus Pd estimation (A) and mammary glands Pd (B) estimation by the NRC (2012) gestating sow model. The model was parameterized to represent a gilt weighing 140 kg at breeding (12.5 pigs/litter, 1.4 kg birth weight) fed 2.21 kg/d between breed to d 89 and 2.61 kg/d from d 90 to farrowing

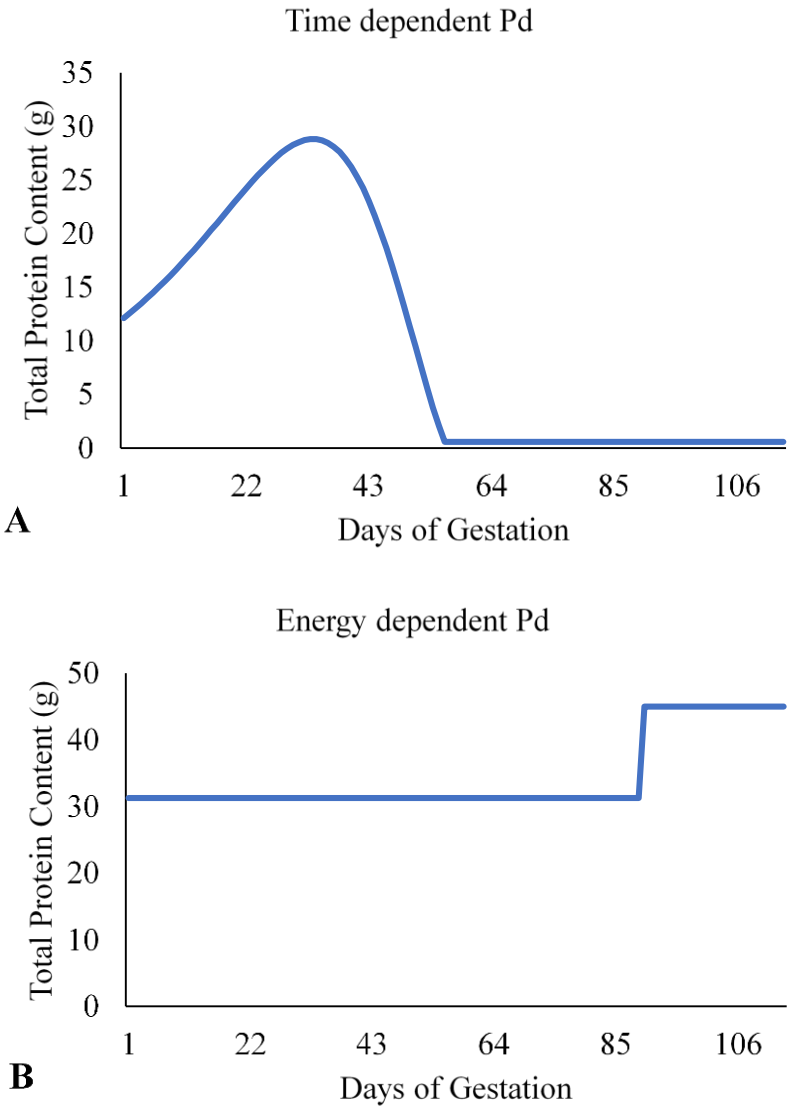

Figure 1-3. Time dependent Pd (A) and energy dependend Pd (B) estimation by NRC (2012) gestating sow model. The model was parameterized to represent a gilt weighing 140 kg at breeding (12.5 pigs/litter, 1.4 kg birth weight) fed 2.21 kg/d between breed to d 89 and 2.61 kg/d from d 90 to farrowing.

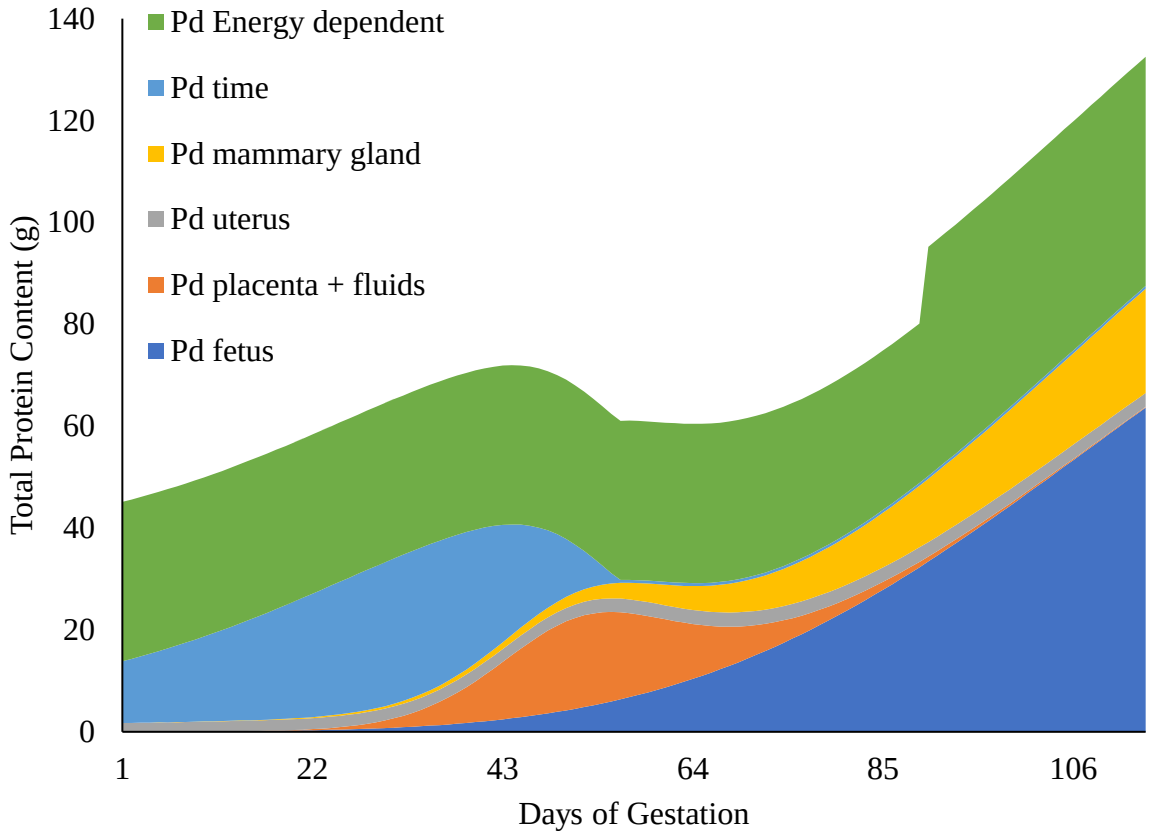

Figure 1-4. Summary of the estimation of the various protein pools according to NRC (2012) gestating sow model. The model was parameterized to represent a gilt weighing 140 kg at breeding (12.5 pigs/litter, 1.4 kg birth weight) fed 2.21 kg/d between breed to d 89 and 2.61 kg/d from d 90 to farrowing.

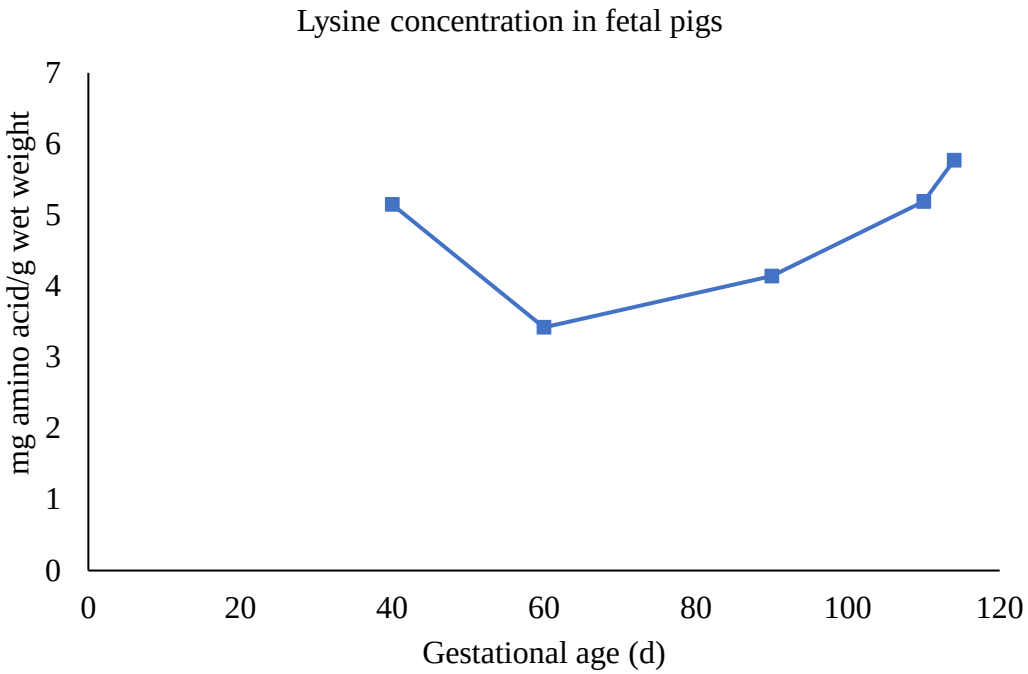

Figure 1-5. Lysine N in the fetal pig. Lys N concentration in fetal pigs increased ($P < 0.05$) progressively from d 60 to 114 of gestation, as analyzed by polynomial regression analysis [adapted from Wu et al. (1999)].

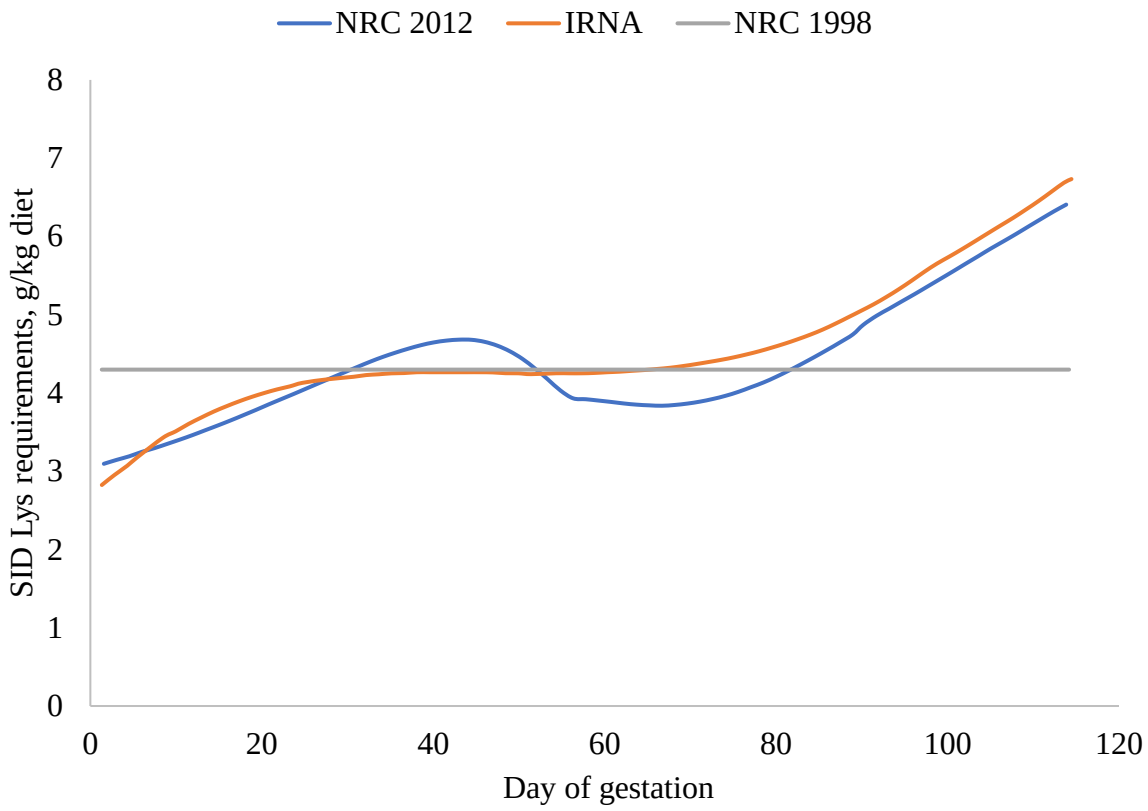

Figure 1-6. SID Lys requirements for pregnant sows estimated by the NRC 1998, NRC 2012 and InraPorc models. These models were parameterized to represent similar conditions. A second parity sow weighing 165 kg at breeding (13.5 pigs/litter, 1.4 kg birth weight) fed 2.21 kg/d. For the NRC 1998 model true ileal digestibility values were transformed to standardized ileal digestibility.

CHAPTER 2

Efficiency of standardized ileal digestible Lys utilization for whole body protein deposition in pregnant gilts and sows during early, mid and late gestation[1]

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2.1. Abstract

NRC (2012) gestating sow model assumes the efficiency of AA use is constant across gestation, which may not reflect changes in metabolic demand during gestation. Efficiency of utilization is determined as the slope of the response to graded levels of test AA. Previous work reported a lack of response to graded Lys [60 – 90% of NRC (2012) predicted requirement] in early and mid gestation. Therefore, a study was conducted to determine the efficiency of SID Lys utilization for whole body protein deposition (kSID Lys WB Pd) in gilts and sows during early (d 48-52), mid (d 75-79) and late gestation (d 103-107). Four isocaloric (3,373 kcal ME/kg) and isoproteic (12.75 % CP) diets containing 40, 50, 60, and 70% of NRC (2012) model-predicted daily SID Lys requirement (10.1 and 9.3 g/d in early and mid gestation, respectively) were randomly assigned to 50 females (PIC 1050; 12 gilts, 21 Parity 1, 17 Parity 2). Dietary

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indispensable AA contents were set to meet 100 – 200% of AA:Lys ratios. Whole body nitrogen (N) retention was based on N balance studies in early, mid and late gestation (7-d diet adaptation and 5 d total urine collection and grab fecal sampling). The kSID Lys WB Pd was determined by simple linear regression and reproductive performance data were analyzed using PROC MIXED procedure of SAS. Comparisons between kSID Lys WB Pd were performed by a Potthoff (1966) analysis using the PROC GLM procedure of SAS. A linear relationship was detected for all parities and stages of gestation, except for late gestation in gilts which showed a trend towards significance (P=0.056). The data for mid gestation in P2 sows was removed due to data failure to meet statistical assumptions (i.e. normality of residuals). Reproductive performance of sows (birth weight, born alive, stillborn, and mummies) was not different by parity or diet. The kSID Lys WB Pd for gilts was 0.71, 0.37 and 0.52 and for P1 sows was 0.56, 0.38 and 0.48 for early, mid and late gestation, respectively. The kSID Lys WB Pd for P2 sows was 0.44 and 0.65 for early and late gestation, respectively. The kSID Lys WB Pd is not constant across stage of gestation and parity.

Keywords: lysine efficiency, parity, gestation stage, pregnant sows, protein retention

2.2. Introduction

Within the concept of precision feeding, an estimation of protein utilization efficiency is important to determine whole body AA requirements to more closely account for all metabolic demands of AA and limit the excretion of excess AA. The kSID Lys WB Pd is a coefficient utilized in the estimation of the SID Lys requirements during pregnancy by the NRC (2012) gestating sow model and the InraPorc model. It is assumed that kSID Lys WB Pd is constant throughout gestation and does not vary among parities (NRC, 2012; van Milgen and Dourmad, 2015). Because the SID Lys requirement varies with age and because different types of tissues with different AA profiles are formed across pregnancy the hypothesis of the present study is that the kSID Lys WB Pd differs across stages of gestation and parities. A better understanding of the kSID Lys WB Pd would help to improve the model to predict SID Lys requirement during pregnancy.

2.3. Materials and Methods

The experiment protocol was approved by the South Dakota State University Animal Care and Use Committee (17-012A) and followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Third Ed., 2010). The experiment was conducted in four blocks, the first block was conducted from September 2017 to January 2018, the second block from November 2017 to February 2018, the third block from January 2018 to May 2019 and the fourth block from May 2019 to August 2019.

2.3.1. **Animals and management**

The experiment was conducted at South Dakota State University Swine Education and Research Facility, Brookings, SD. A total of 50 females (PIC 1050; 12 gilts, 21 Parity 1, 17 Parity 2sows; 189 ± 22.04 kg at d 42 ±1 of gestation) in 2 blocks were used in the experiment. Females were kept in gestation stalls (61 cm x 1.98 m) from breeding to 110 d of gestation and were offered a common gestation diet (3300 kcal/kg ME and 0.46% SID Lys) at a feed allocation per day (i.e. 2.27 kg/d) to maintain a target body condition score of 3.

From d 110 of gestation until weaning at d 21 of lactation, females were housed in farrowing crates (1.83 m x 2.44 m.) and offered a common lactation diet (3300 kcal/kg ME and 0.64% SID Lys), according to standard feed curve protocol of the swine unit. Lactation feed was administered by an electronic feeding system (Gestal 3G; Jyga Technologies, Greeley, KS, USA) allowing daily intake up to 20% above the set curve for ad libitum intake. Gestation diets were provided in meal form and water was provided ad libitum.

Pigs and facilities were checked twice daily by trained research unit manager and assistant manager and by the assigned graduate research assistant during the N balance periods.

2.3.2. **Experimental design and dietary treatments**

When confirmed pregnant, animals were randomly assigned to one of 4 experimental diets balancing as best as possible for parity and BW within block. The SID Lys levels in the experimental diets **Lys-1, Lys-2, Lys-3**, and **Lys-4** were set to provide 40, 50, 60, and 70%, respectively, of the model-predicted daily SID Lys for protein

retention (NRC, 2012) for a parity 1 sow, in each of period I (d 41-52, 10.1 g/d), period II (d 68-79, 9.3 g/d) and period III (d 96-107, 15.56 g/d). Experimental diets were given at a rate of 2.21 kg/d during periods I and II; and at 2.61 kg/d during period III according to the NRC (2012) recommendation for parity 1 gestating sows in two equal meals (i.e. 0630 and 1430 h). The formulated SID Lys levels of the experimental diets are shown in Table 2-1. Diets were formulated to contain 3370 kcal/kg ME, 12.6 % CP, 0.8% total calcium and 0.35% available P. To set the nutrient specifications of the experimental diets, High-Lys (0.26 %) and Low-Lys (0.52 %) master diets were blended (Table 2-2). The ingredient composition and nutrient content of the two master diets are presented in Table 2-3.

The SID AA: SID Lys ratios were 85-110% higher than the recommendations of NRC (2012). Essential SID AA levels other than Lys were at 2-72% overage to ensure that other essential AA were not limiting the response. Titanium dioxide at 0.20% was used as an indigestible marker to calculate total tract N digestibility.

2.3.3. **General observations**

Body weight of the females were measured before and within 24-h after each N balance period for the determination of daily SID Lys requirements for maintenance and for gestation weight gain. At farrowing, the number of piglets born alive, still births and mummified fetuses were recorded. Daily feed disappearance was monitored for feed spillage and feed refusal. Sow illness, lameness, reproductive failure and mortality and clinical signs of infection over the course of catheterization were noted.

2.3.4. **Nitrogen Balance and sample analysis**

In each block, three 12 d N balance periods were conducted starting at d 41, 68 and 96 of gestation. Each period consisted of 7 d diet adaptation and 5 d total urine collection and grab fecal sampling daily. Nitrogen balance observations were based on total urine collection using Foley catheters and determination of fecal N digestibility using indigestible marker. Urine was collected as described by Miller et al., (2016). Prior to each collection, urinary catheters (Lubricath, 2-way, 30 mL balloon, 18 French; Bard Medical Division, Covington, GA, USA) were lubricated and inserted flaccidly through the urethra and the balloon was inflated with 30 mL saline solution to retain the catheter in the bladder. Catheters were connected to closed containers using polyvinyl tubing (Fisherbrand Clear PVC Tubing, 4.88 mm inner diameter; Fisher Scientific Co., Birmingham, AL, USA) and urine collected. Sulfuric acid was added to the containers to maintain pH <3. A representative subsample (1% of the successful daily collection) were obtained, pooled within each collection period and stored at 4 °C until further analysis. Urine collection for each balance period was considered successful when at least 3 d of collections were accomplished. Urinary catheters were removed at the end of each N-balance period. Fecal samples were obtained by rectal palpation and daily collections were pooled per gilt and period and stored at -20 °C until further analysis.

Subsamples of all feed used was collected, pooled and homogenized per period and block. Approximately 200 g of each experimental diet and freeze-dried feces were ground using rotor mill (Centrifugal Mill ZM 200; Retsch GmbH, Haan, Germany) with 0.50 mm sieve. Urine, freeze-dried feces and experimental diets were analyzed for N content using combustion method (Rapid N III, Elementar Analysensysteme, GmbH,

Hanau, Germany) and CP was calculated as N x 6.25. Dry matter and titanium dioxide content in feces and feeds were quantified according to Short et al. (1996). Absorbance of standard and samples were read using Spectra MAX 190 plate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) at 408 nm wavelength. The AA concentrations and proximate compositions of all the diets were completed by a commercial laboratory (ESCL, University of Missouri, Columbia, MO).

2.3.5. **Calculations**

Efficiency can be defined as output/input; in this regard, the kSID Lys WB Pd was defined as the ratio of whole body SID Lys retention (g/d) to SID Lys intake (g/d) and calculated as the slope of the linear relationship between SID Lys retention (g/d) and SID Lys intake (g/d) as shown in Fig. 2-1. For the calculation of kSID Lys WB Pd, the intercept of the regression model was forced through zero, as at zero intake, Lys retention is assumed to be zero. The kSID Lys WB Pd was calculated by parity (gilts, parity 1 and parity 2+ sows) and stage of gestation (early, mid and late gestation). Lysine retention was calculated according to NRC (2012) gestating sow model (Eq. 8-56 to 8-60), dividing the protein retained (N retained x 6.25) into the six different protein pools and estimating Lys content in each pool and based on actual litter size (including stillborn) and actual piglet birth weight. N retention (g/d) was calculated as daily N intake minus daily N excretion in feces and urine. Fecal N excretion (g/d) was calculated from N intake and total tract N digestibility, with the latter estimated using indicator method (NRC, 2012).

Daily SID Lys intake was calculated as the product of daily feed intake (kg/d), Lys level of diet (g/kg) and SID coefficient (%). SID coefficients were calculated considering the inclusion of corn, soybean meal, and synthetic AA for each diet and their respective digestibility coefficients according to NRC (2012).

2.3.6. **Statistical analysis**

Reproductive performance data were analyzed as unbalanced randomized complete block with diet and parity as the fixed effect and group (i.e. block) as the random effect using the PROC MIXED procedure of SAS (Version 9.3; SAS Inst. Inc., Cary, NC). Differences among treatments were separated using PDIFF option with adjusted Tukey's test. The linear and quadratic response in N retention variables were tested within each balance period using the PROC MIXED procedure of SAS.

The kSID Lys WB Pd was determined by linear regression using PROC MIXED procedure of SAS with litter weight as random effect. Comparisons between slopes were made by a Potthoff (1966) analysis using PROC GLM procedure of SAS. Weaver and Wuensch (2013) have provided SAS code for conducting a Potthoff analysis and complete details on this method. For all analyses, a $P < 0.05$ was considered significant.

2.4. Results

The results are based on data collected and analyzed from block 1 and 2, representing 50 females. From these 50 animals, seven sows suffered abortions during the experimental period, two abortions occurred outside a collection period. The causes of these abortions are not believed to be related to the experimental diets. Two abortions

occurred on day two of N balance 3 in block 2, so further data collection was terminated. The maximum number of observations was 150 (50 animals multiplied by three different stages of gestation) from which 21 observations (7 animals multiplied by 3 stages of gestation) were not collected due to abortions, 22 observations were not collected due to termination of N balance 3 in block 2, 10 observations were not collected due to catheter expulsion and unsuccessful catheterization, 1 observation was not collected due to signs of sickness and refusal to eat, and 3 observations were removed from the dataset because they were considered outliers. The total number of successful observations were 93; 17 for gilts (8 for early, 7 for mid, and 2 for late gestation), 46 for P1 sows (18 for early, 16 for mid, and 12 for late gestation), and 30 for P2 sows (13 for early, 12 for mid, and 5 for late gestation).

2.4.1. **Experimental Diets**

Two master diets were blended to obtain diets that matched 40, 50, 60 and 70% of the Lys requirement above maintenance in each balance period for parity 1 sows as shown in Table 2-2 The ingredient composition and nutrient content of the two master diets are presented in Table 2-3. Table 2-4 shows the analyzed SID Lys content of the diets and the percentage of the SID Lys requirement above maintenance by parity and stage of gestation. For all parities, the SID Lys content of the experimental diets was below the SID Lys requirement generated from NRC (2012) model. There was a maximum of 4% difference between the diets formulated versus those analyzed. The ratios of other essential AA to SID Lys were 30-70% above the NRC (2012) recommendation for ideal ratio and thus unlikely to be limiting response to dietary Lys.

2.4.2. **Nitrogen Balance**

Whole body N retention was affected by experimental dietary levels of SID Lys, indicating that the levels of Lys limited protein deposition in the three stages of gestation (early, mid and late) for gilts and sows. Table 2-5 shows the N utilization variables by diet and parity. Figure. 2-2, Fig. 2-3 and Fig. 2-4 show the linear relationship between SID Lys intake (g/d) and SID Lys retention for WB Pd (g/d) by stage of gestation for gilts, parity 1 and parity 2, respectively. A linear relationship was detected for all parities and stages of gestation, except for late gestation in gilts which showed a trend towards significance (P=0.056). It is believed the linear relationship between SID Lys intake and SID Lys retention in gilts in late gestation is not significant because there are only two observations, rather than because there is not a true linear relationship, thus, the results of late gestation in gilts are still reported in the present document. In order to account for this lack of statistical significance and for strengthening the dataset 38 additional animals (divided in block 3 and 4 with 17 and 21 animals respectively) were included into the experiment for potentially 114 new observations. At the writing of this report samples from these 38 animals have been collected and are currently being analyzed. P1 sows that were part of this group of 38 animals were not fed the lowest level of Lys because a protein sparing effect was suspected at this Lys level. With limited availability of P1 sows, the use of the 3 levels of Lys is expected to add a greater degree of robustness to the data set.

Additionally, the data corresponding to P2 sows for mid gestation were excluded from the dataset since despite existing a significant P-value for the linear regression analysis between SID Lys intake and SID Lys retention (P<0.001) the assumption of

normality of the residuals was violated. It is believed that the P-value of the regression analysis for P2 sows in mid gestation was significant only because it was regressed through zero but there was not a true linear relationship as the Shapiro-Wilk test of normality revealed (P=0.014).

The kSID Lys WB Pd was greatest in early gestation for gilts and late gestation for P2 sows and lowest for all parities in mid gestation (Fig. 2-5). Average kSID Lys WB Pd for early, mid and late gestation across all parities was 0.55, 0.37 and 0.53, respectively.

2.4.3. **Farrowing Performance**

The numbers of total born, born alive, stillborn piglets, and mummies as well as the average piglet weight at birth were not affected by Lys level of the diets. The results of the ANOVA for the farrowing performance by diet and parity is shown in Table 2-7. Total born was defined as the number of piglets born alive and stillborns.

2.5. Discussion

The objective of the current project was to determine the kSID Lys WB PD in gestating sows as influenced by age and stage of pregnancy. The linear response between graded daily SID Lys intake below predicted requirements and estimated SID Lys retention was used as the tool on which to determine the kSID WB Pd for different parities (gilts, P1 and P2 sows) and stages of gestation (early, mid and late). Daily SID Lys intake levels were set below the requirement because at the requirement, linear regression reaches a plateau in which additional SID Lys intake does not result in an incremental increase in SID Lys retention. The intent was to avoid the plateau zone and

the transition zone between the linear and plateau phases. According to Pomar et al. (2003) the length and extent of the transition zone differs between populations and increases with the variability of the population. There are currently no studies reporting the range of percentages that would define the transition zone for sow SID Lys requirements during pregnancy. The present study aimed to target SID Lys levels outside the transition and plateau zone based on the results obtained by Navales et al. (2018).

Navales et al. (2018) reported that the assumption of consistent kSID Lys WB Pd during pregnancy (NRC, 2012) does not reflect changes in metabolic demands. Navales et al. (2018) provided gilts with SID Lys deficient diets ranging from 60 to 90% of the daily SID Lys requirement above maintenance according to the NRC (2012) gestating sow model during early (d 48 to d 52), mid (d 675 to d 79), and late gestation (d 103 to 107). Navales et al. (2018) found no linear relationship between SID Lys intake and SID Lys retention for early and mid gestation and found a significant linear relationship for late gestation. The SID Lys levels chosen for the present study were lower than the levels used by Navales et al. (2018) for gilts and P1 sows and similar for P2 sows. A linear relationship was detected for all parities and stages of gestation except for P2 sows in mid gestation.

Another intent for the determination of the kSID Lys WB Pd was to avoid the protein sparing effect zone. At low SID Lys intake levels, the body derives energy from sources other than protein in order to conserve skeletal tissue. In the current study, the increase in SID Lys retention in early gestation at the lowest SID Lys level for P1 sows may indicate a protein sparing effect. The biological reason for this increase is not clear as a similar protein sparing effect was not observed for gilts or P2 sows in early gestation.

The kSID Lys WB Pd of pregnant sows has been reported as a constant coefficient regardless of parity and gestation stage (Everts and Dekker, 1995; NRC, 2012); however, the findings of the current study suggest that the kSID Lys WB Pd varies by parity and gestation stage. The changes in kSID Lys WB Pd throughout gestation are presumed to be related to physiological differences in the various tissues that make up the whole body of the gestating sow, some of which also begin to demand proteins at distinct points during pregnancy.

In early gestation placenta and allantoic fluid are the primary products of conception that are being formed (Knight et al., 1977; Wu et al., 2005; NRC, 2012). During the formation of placenta and allantoic fluid, AA biosynthesis should occur; approximately 50% of the total free AA present in allantoic fluid are arginine and ornithine and it is believed that allantoic fluid is a reservoir of nutrients (Wu et al., 2010). Arginine and ornithine can be biosynthesized through the urea cycle from the N from other AA like Lys. Therefore, the efficiency of SID Lys utilization for conceptus Pd may increase during placenta and allantoic fluid Pd due to the biosynthesis of arginine and ornithine and could explain a greater kSID Lys WB Pd in early gestation compared to mid gestation.

In mid gestation pregnant sows have a primary need for energy rather than AA to support late gestation energy requirements (Samuel, 2008) and the allantoic fluids produced in early gestation may act as a reservoir of AA that fetal development demands at this point. A reduction in AA demand for conceptus Pd in combination with an increase in maternal energy demand could explain the reduction of kSID Lys WB Pd in mid gestation compared to early gestation.

In late gestation, the fetus and mammary glands are the primary products of conceptus in development (NRC, 2012). Fetal protein accretion rate increases after d 69 of gestation and mammary protein accretion mainly occurs after day 82 of gestation (Kim et al., 2005). In the fetus and mammary glands, AA utilization is influenced by the activities of the AA transporters which are affected by maternal blood flow (Kim and Wu, 2009; Zhang et al., 2018). Blood flow is an important determinant of substrate concentration at the site of transport (Lewis et al., 2013). Maternal blood flow and uterine blood flow increases as gestation progresses and are closely related to fetal growth and litter size (Père and Etienne, 2000). Thus, this increase in blood flow during late gestation increases the activity of the AA transporters and could account for the increase in the kSID WB Pd during late gestation compared to mid gestation.

Regarding the differences in kSID Lys WB Pd by parity, the observed kSID Lys WB Pd in early gestation was greater for gilts followed by P1 sows and P2 sows, these results are consistent with results obtained from growing-finishing pigs experiments which revealed that the marginal efficiency of using SID Lys for body Pd decline with increasing BW (NRC, 2012).

In late gestation, the differences in the kSID Lys WB Pd between parities may be linked to changes in the efficiency of SID Lys utilization for conceptus Pd. As gestation progresses insulin resistance increases possibly for the purpose of activating the accumulative transporters in the placenta (Broer, 2008) and mammary gland (Zhang et al., 2018). Increasing insulin resistance would therefore reduce maternal Pd and boost conceptus Pd as gestation progresses. There are reports that suggest that there are differences in insulin resistance by parity. Barbour et al. (2007) stated that TNF-α is a

predictor of insulin resistance both in animals and humans and primiparous sows have lower plasma concentrations of TNF-α than multiparous sows (Ison et al., 2018); this would indicate that gilts are less insulin resistant compared to older sows. Moreover, Balzani et al. (2016) reported that gilts and P1 sows had less developed mammary glands compared with older multiparous sows. Thus, the higher kSID Lys WB Pd noted for P2 sows compared with P1 sows and gilts in late gestation may be linked to the increased AA demand for mammary glands in P2 sows and a lower maternal Pd influence due to the development of insulin resistance.

In addition, litter size has a positive correlation with blood flow (Père and Etienne, 2000), as earlier mentioned, and blood flow impacts AA transporter activity (Lewis et al., 2013). Differences in litter size by parity may play a role in the difference in the kSID Lys WB Pd in late gestation and may explain difference in the kSID Lys WB Pd between P2 sows and gilts.

In conclusion, the kSID Lys WB Pd varies by parity and stage of gestation. These differences in kSID Lys WB Pd suggest the need to review the SID Lys requirements models for pregnant sows and to develop strategies for increasing the kSID Lys WB Pd. Also, the differences in kSID Lys WB Pd by parity and stage of gestation suggest the need for parity-segregated phase feeding of pregnant sows.

Table 2-1. Formulated SID Lys levels for daily intake of P1 sows by treatment.

	Parity 1 sows		
	Early gestation (d 41 to 52)	**Mid gestation (d 68 to 79)**	**Late gestation (d 96 to 107)**
Feed Allocation, kg/d	2.21	2.21	2.61
SID Lys Requirement[1], g/d	10.1	9.3	15.56
SID Lys Requirement for Maintenance[2], g/d	1.79	1.89	1.99
SID Lys Requirement above Maintenance[3], g/d	8.31	7.41	13.57
SID Lys Levels by Treatment[4], g/d			
Lys-1	5.11	4.85	7.42
Lys-2	5.94	5.59	8.77
Lys-3	6.78	6.34	10.13
Lys-4	7.61	7.08	11.49

[1] NRC-generated SID Lys requirements settled as follows:
P1 sows: BW at breeding = 165 kg; parity = 2; gestation length = 114 d; anticipated litter size = 13.5; anticipated birth weight = 1.4 kg/pig, and feed intake = 2.21 kg/d at d 1 - 90 of gestation and 2.61 kg/d at d 90-110 of gestation.
[2] SID Lys requirements for maintenance were estimated with the factor 34.79 mg/kg BW^0.75 for expected BW (NRC, 2012).
[3] SID Lys requirements above maintenance were calculated as: (SID Lys Requirement, g/d - SID Lys Requirement for Maintenance, g/d)
[4] SID Lys consumed daily by treatment group considering the SID Lys content of the diets and the allocation. Lys-1, Lys-2, Lys-3, and Lys-4 consumed 40, 50, 60 and, 70% of the SID Lys requirement above maintenance, respectively. SID Lys levels consumed daily by treatment were calculated as follows: [(SID Lys Requirement above maintenance, g/d * percentage required for each treatment) + SID Lys Requirement for Maintenance[2], g/d]

Table 2-2. Levels of the master diets inclusion in the 12 experimental diets.

	Early[5]				Mid[5]				Late[5]			
	Lys-1	Lys-2	Lys-3	Lys-4	Lys-1	Lys-2	Lys-3	Lys-4	Lys-1	Lys-2	Lys-3	Lys-4
Master diets inclusion, %												
Low-Lys	94.3	77.3	60.2	43	100	84.6	69.6	54.5	70.6	47.3	23.5	-
High-Lys	5.7	22.7	39.8	57	-	15.4	30.4	45.5	29.4	52.7	76.5	100

[5] Stage of gestation: Early (d 41 to 52); Mid (d 68 to 79); Late (d 96 to 107).

Table 2-3. Ingredient composition and nutrient content of Low + High Lys Master diets.

Items	Master Diets	
	Low	**High**
Ingredients, %		
Corn	86.35	81.40
Soybean Meal, 46%	2.70	11.80
Soybean Oil	-	2.00
Glutamic Acid	6.75	-
DL-Methionine	0.05	0.27
L-Threonine	0.16	0.33
L-Tryptophan	0.04	0.08
L-Valine	0.06	0.23
L-Isoleucine	0.03	0.10
L-Phenylalanine	-	0.08
Titanium Dioxide	0.20	0.20
Others[6]	3.84	3.72
Calculated Nutrient Content		
ME, kcal/kg	3,370	3,370
Crude Protein, %	12.6	12.9
Total Lys, %	0.29	0.54
SID Lys, %	0.23	0.45
Ratio to SID Lys		
SID Met+Cys	1.52	1.41
SID Thr	1.69	1.5
SID Trp	0.43	0.41
SID Val	1.65	1.52
Total Ca, %	0.80	0.80
Dig. P, %	0.35	0.35
Analyzed Nutrient Content		
Crude Protein, %	11.40	11.70
Total Lys, %	0.30	0.54
SID Lys[7], %	0.23	0.45
Ratio to SID Lys		
SID Met+Cys	1.18	1.19
SID Thr	1.29	1.20
SID Trp	0.30	0.35
SID Val	1.33	1.37

[6] Other (% inclusion): Calcium carbonate: 1.45 and 1.39 in Low and High Master diets, respectively; MCP: 1.49 and 1.43 in Low and High Master diets, respectively; salt: 0.50; mineral premix: 0.15 which provided the following per kilogram of diet: 33000 IU vitamin A,33000 IU vitamin D3, 284 IU vitamin E, 0.132 mg vitamin B12, 13 mg menadione, 30 mg riboflavin, 99 mg D-panthothenic acid, 165 mg niacin, 13 mg folic acid, 45 mg pyridoxine, 10 mg thiamine, 1 mg biotin; and vitamin premix: 0.05 which provided the following per kilogram of diet: 6 mg Zn as ZnSO4, 6 mg Fe as FeSO4; 0.5 mg Cu as CuSO4, and 1.5 mg Mn as MnSO4.

[7] There was a 3% difference between calculated SID Lys content and analyzed SID Lys content; the difference cannot be seen because only two decimals are displayed.

Table 2-4. Feed allocation and SID Lys (g/d) of experimental treatments.

	Gilts			Parity 1 sows			Parity 2 sows		
Stage of Gestation[8]	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**
Feed Allocation, kg/d	2.21	2.21	2.61	2.21	2.21	2.61	2.21	2.21	2.61
SID Lys Reqt[9], g/d	12.00	11.10	17.7	10.1	9.3	15.56	8.3	7.5	13.2
SID Lys Reqt for Maint[10], g/d	1.61	1.71	1.82	1.79	1.89	1.99	2.06	2.16	2.25
SID Lys Reqt above Maint[11], g/d	10.39	9.39	15.88	8.31	7.41	13.57	6.24	5.34	10.95
SID Lys Levels by Treatment[12], g/d, %									
Lys-1	5.39	36%	5.11 36% 7.7 37%	5.39	43%	5.11 44% 7.7 42%	5.39	53%	5.11 55% 7.7 50%
Lys-2	6.2	44%	5.84 44% 9.01 45%	6.2	53%	5.84 53% 9.01 52%	6.2	66%	5.84 69% 9.01 62%
Lys-3	7.01	52%	6.57 52% 10.35 54%	7.01	63%	6.57 63% 10.35 62%	7.01	79%	6.57 83% 10.35 74%
Lys-4	7.84	60%	7.29 59% 11.68 62%	7.84	73%	7.29 73% 11.68 71%	7.84	93%	7.29 96% 11.68 86%

[8] Stage of gestation: Early (d 41 to 52); Mid (d 68 to 79); Late (d 96 to 107).

[9] NRC-generated SID Lys requirements settled as follows:

Gilts: BW at breeding = 140 kg; parity = 1; gestation length = 114 d; anticipated litter size = 12.5; anticipated birth weight = 1.4 kg/pig, and feed intake = 2.21 kg/d at d 1 - 89 of gestation and 2.61 kg/d at d 90-110 of gestation.

Parity 1 Sow: BW at breeding = 165 kg; parity = 2; gestation length = 114 d; anticipated litter size = 13.5; anticipated birth weight = 1.4 kg/pig, and feed intake = 2.21 kg/d at d 1-89 of gestation and 2.61 kg/d at d 90 - 110 of gestation

Parity 2 Sow: BW at breeding = 185 kg; parity = 3; gestation length = 114 d; anticipated litter size = 13.5; anticipated birth weight = 1.4 kg/pig, and feed intake = 2.21 kg/d at d 1-89 of gestation and 2.61 kg/d at d 90 – 110 of gestation

[10] SID Lys requirements for maintenance were estimated with the factor 34.79 mg/kg BW^0.75 for expected BW (NRC, 2012).

[11] SID Lys requirements above maintenance were calculated as: (SID Lys Requirement, g/d - SID Lys Requirement for Maintenance, g/d)

[12] SID Lys consumed daily by treatment group considering the SID Lys content of the diets (obtained by AA profile analysis) and the allocation. The percentages were calculated as follows: [(SID Lys consumed daily by treatment group – SID Lys requirement for maintenance, g/d)/SID Lys requirement above maintenance, g/d]

Table 2-5. N utilization variables by diet and parity.

	Diets						Linear		Quadratic		Parity[13]			Linear[14]	
	Lys-1	**Lys-2**	**Lys-3**	**Lys-4**	**SE**	**P-value**	**SE**	**P-value**	**0**	**1**	**2**	**SE**	**P-value**
Early gestation[15]													
N-intake, g/d	40.37	40.54	40.70	40.87					40.62	40.62	40.66		
Fecal N, g/d	4.15	4.54	4.65	4.25	0.07	0.347	0.34	0.001	4.54	4.42	4.32	0.09	0.221
Urine N, g/d	24.54	24.41	21.55	21.75	0.59	0.021	3.28	0.924	20.91	22.75	25.13	0.69	0.004
N retention[16], g/d	11.68	11.59	14.50	14.87	0.56	0.009	3.14	0.772	15.17	13.46	11.13	0.68	0.004
N digestibility[17], %	89.73	88.80	88.57	89.6	0.18	0.519	0.84	0.001	88.84	89.13	89.36	0.21	0.232
N efficiency[18], %	28.94	28.59	35.62	36.38	1.39	0.012	7.74	0.777	37.31	33.13	27.41	1.65	0.004
Mid gestation[15]													
N-intake, g/d	40.31	40.46	40.61	40.76					40.49	40.50	40.57		
Fecal N, g/d	4.20	4.66	4.30	4.40	0.07	0.584	0.31	0.055	4.52	4.33	4.39	0.08	0.479
Urine N, g/d	26.83	24.70	26.09	23.34	0.63	0.094	2.81	0.894	25.65	25.28	25.74	0.68	0.889
N retention[16], g/d	9.28	11.09	10.22	13.03	0.61	0.050	2.75	0.721	10.35	10.91	10.33	0.67	0.908
N digestibility[17], %	89.58	88.47	89.41	89.21	0.18	0.804	0.76	0.054	88.84	89.31	89.16	0.18	0.513
N efficiency[18], %	23.03	27.42	25.16	31.96	1.52	0.061	6.79	0.730	25.51	26.92	25.54	1.65	0.923
Late gestation[15]													
N-intake, g/d	47.95	48.22	48.50	48.78					48.36	48.36	48.36		
Fecal N, g/d	5.52	5.54	5.93	6.84	0.09	0.055	0.55	0.064	6.32	5.93	5.97	0.26	0.676
Urine N, g/d	20.55	21.39	25.41	23.27	0.46	0.031	3.06	0.325	23.55	23.53	20.62	1.30	0.166
N retention[16], g/d	16.61	18.11	22.44	21.84	0.62	0.006	3.13	0.528	18.50	18.93	21.69	1.45	0.199
N digestibility[17], %	88.63	88.65	87.63	85.81	0.19	0.032	1.13	0.064	86.93	87.74	87.63	0.55	0.703
N efficiency[18], %	46.26	44.78	34.64	37.56	0.96	0.008	6.48	0.522	38.23	39.09	44.89	2.92	0.184

[13] Parity 0: Gilts; Parity 1: P1 Sows; Parity 2: P2 Sows.
[14] No significant quadratic effect for parity was detected.
[15] Stage of gestation: Early (d 41 to 52); Mid (d 68 to 79); Late (d 96 to 107).
[16] N retention was calculated as: [N intake – (Fecal N + Urine N)]
[17] Fecal N digestibility determined using indigestible marker.
[18] N efficiency was estimated as: (N retention / N intake)

Table 2-6. Sow farrowing performance by diets and parity.

	Diets				ANOVA		Parity[19]			ANOVA	
	Lys-1	**Lys-2**	**Lys-3**	**Lys-4**	**SEM**	**P-value**	**0**	**1**	**2**	**SEM**	**P-value**
Farrowing performance											
No. of animals	12	11	12	9			8	20	16		
Total litter size[20]	16.58	18.27	15.08	14.22	3.73	0.085	14.00	16.15	17.13	3.83	0.182
Born alive	15.25	17.27	14.33	13.44	3.59	0.107	13.50	14.95	16.18	3.69	0.244
Stillborn	1.33	1.00	0.75	0.78	0.93	0.417	0.50	1.20	0.94	0.91	0.194
Mummified	0.67	0.64	0.50	0.44	0.81	0.908	0.25	0.50	0.81	0.78	0.229
Avg Birth weight	1.44	1.31	1.45	1.41	0.20	0.380	1.33	1.42	1.43	0.20	0.511

[19] Parity 0: Gilts; Parity 1: P1 Sows; Parity 2: P2 Sows.
[20] Total litter size was defined as: born alive + stillborns.

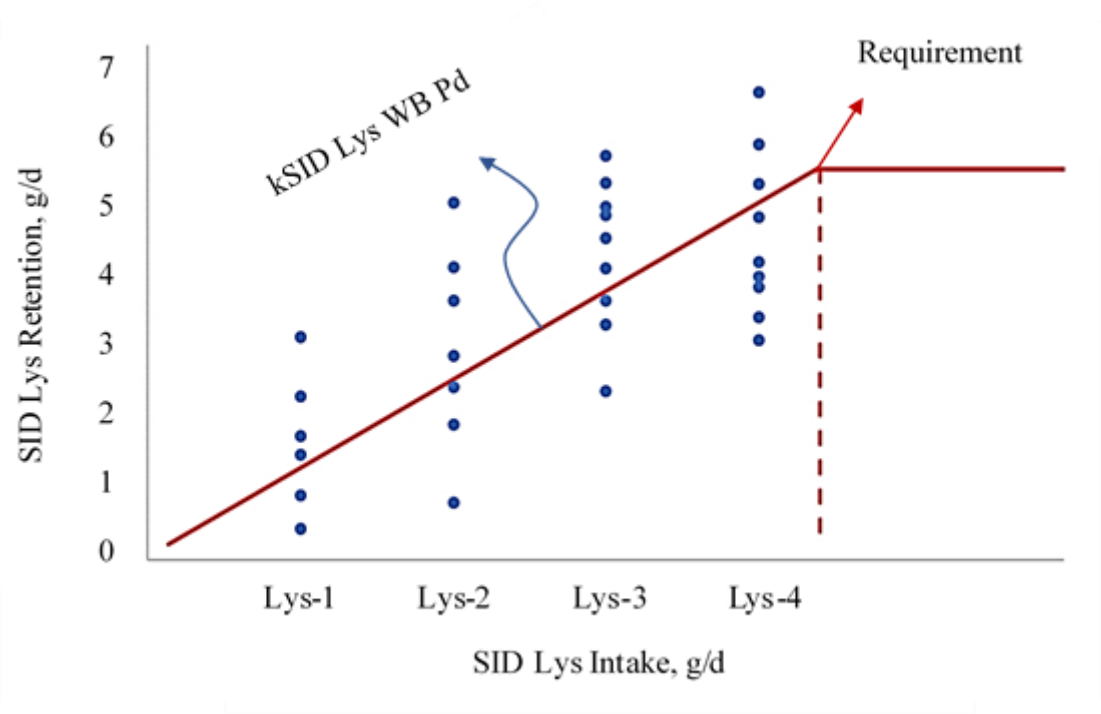

Figure 2-1. The efficiency of SID Lys utilization for WB Pd is the slope of SID Lys Retention

vs. SID Lys Intake

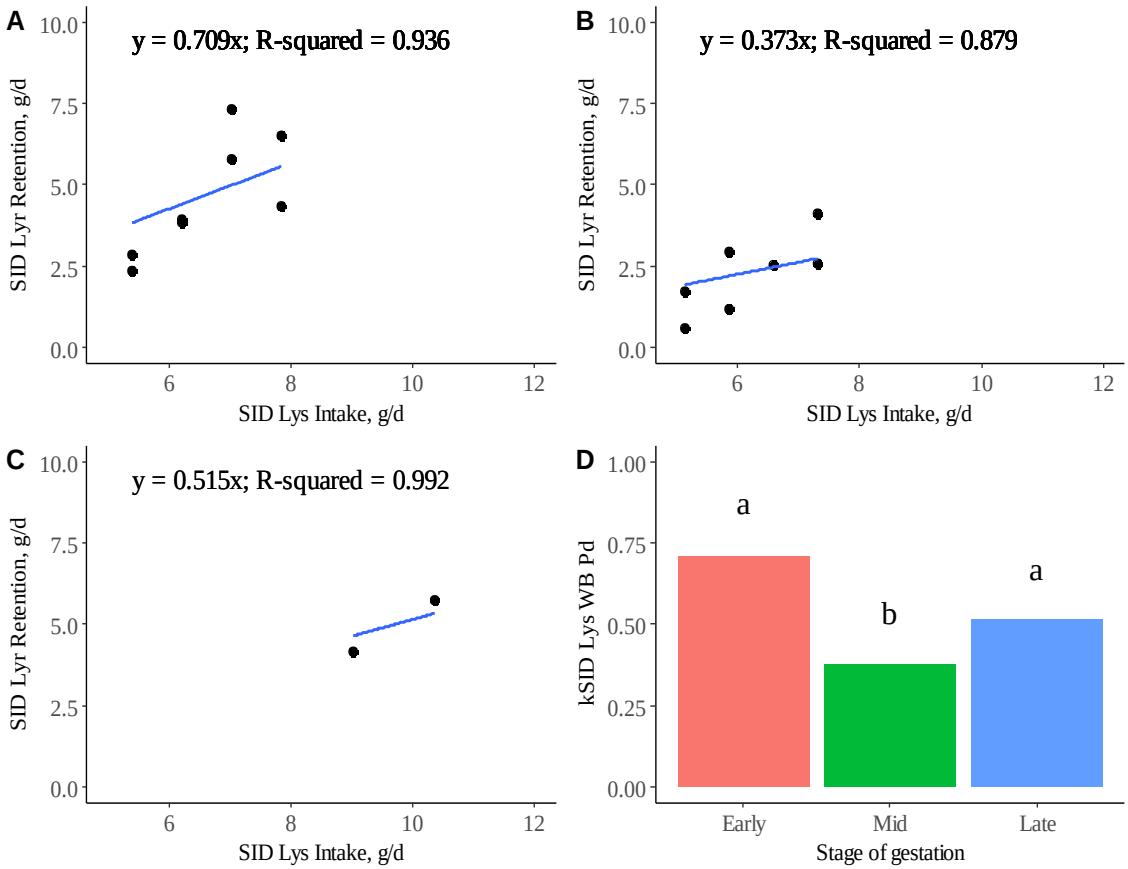

Figure 2-2. The linear relationship between SID Lys intake and SID Lys retention for whole body protein deposition for gilts in early (A), mid (B), and late gestation(C). A compilation of the slopes from early, mid and late gestation is summarized (D). Values with different superscript letters in a column are significantly different ($p<0.05$).

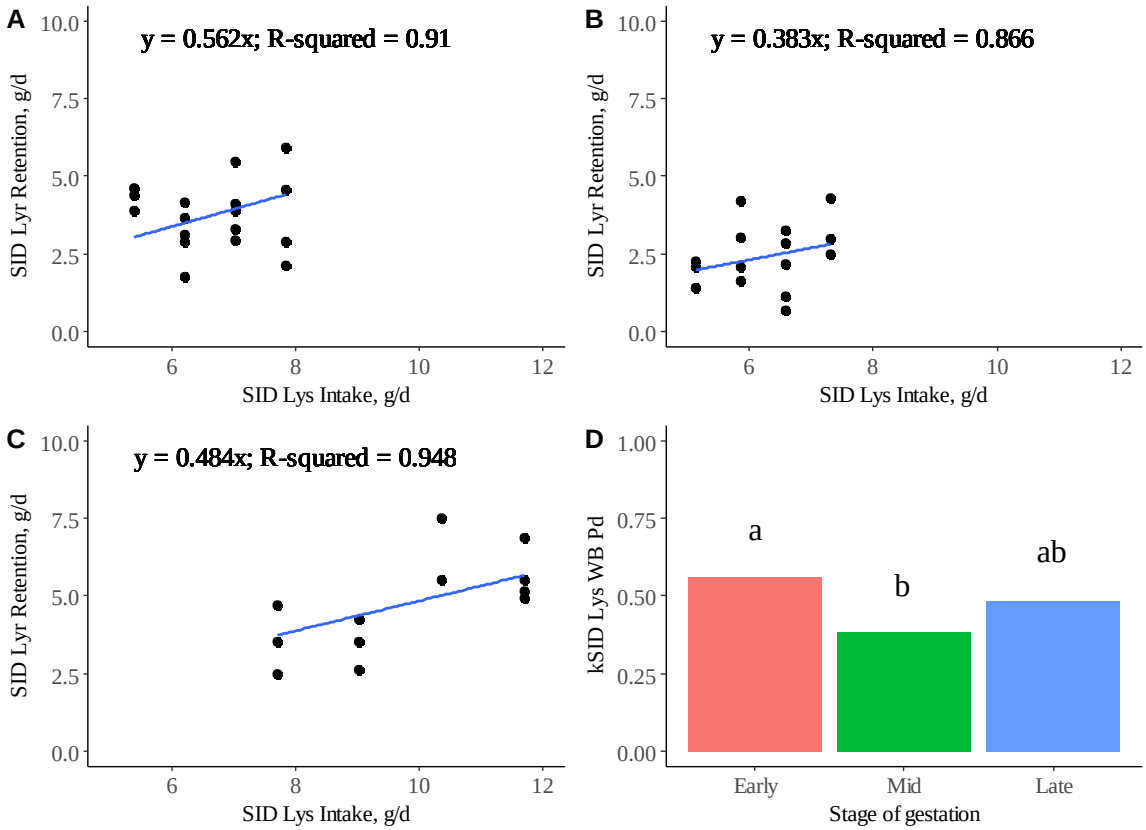

Figure 2-3. The linear relationship between SID Lys intake and SID Lys retention for whole body protein deposition for P1 Sows in early (A), mid (B), and late gestation(C). A compilation of the slopes from early, mid and late gestation is summarized (D). Values with different superscript letters in a column are significantly different ($p<0.05$).

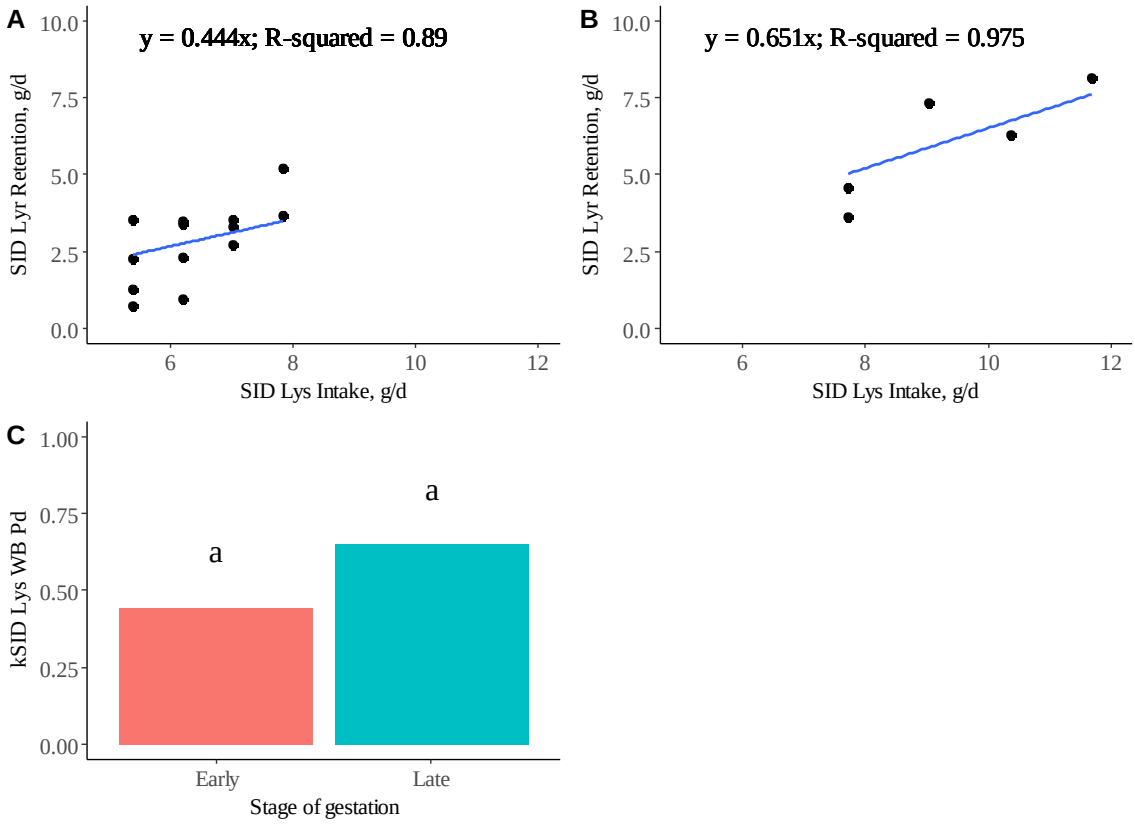

Figure 2-4. The linear relationship between SID Lys intake and SID Lys retention for whole body protein deposition for P2 Sows in early (A) and late gestation (B). A compilation of the slopes from early and late gestation is summarized (C). Values with different superscript letters in a column are significantly different ($p<0.05$).

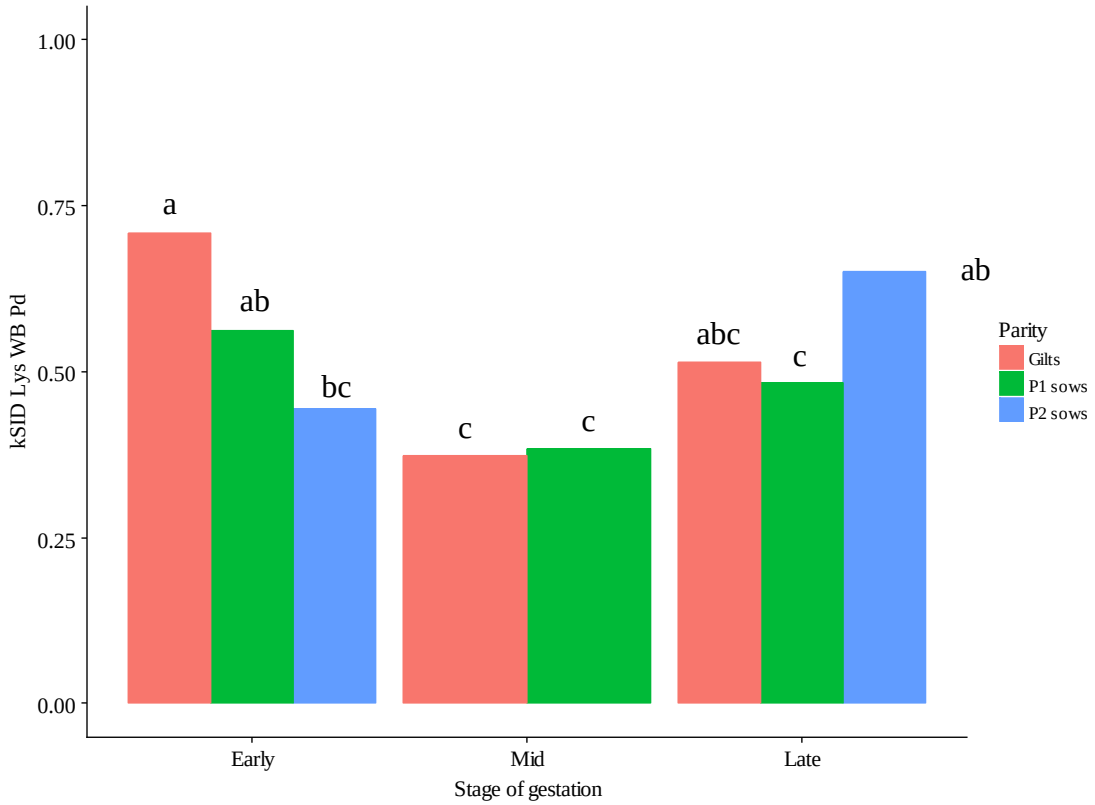

Figure 2-5. The summary of kSID Lys WB Pd for gilts, P1 sows and P2 sows in early (d50) mid (d77) and late gestation (d105). Values with different superscript letters in a column are significantly different ($p<0.05$).

CHAPTER 3

General Discussion

The current study aimed to determine the kSID Lys WB Pd in different parities and stages of gestation. For this purpose, N balance studies were carried out and, from the calculated N retained, the SID Lys retention was estimated with the NRC (2012) gestating sow model equations. Certainly, during the measurement of the N retention and the estimation of the kSID Lys WB Pd there were some random and systematic errors associated. Random effects can be defined as statistical fluctuations in the measured data due to the precision restrictions of the measuring device; while systematic errors can be defined as reproducible inaccuracies that are consistently in the same direction (Taylor, 1997).

In order to account for random effects, a mixed model was used as reported in the Statistical analysis section of Chapter 2. An exploratory experiment was conducted in order to detect possible systematic errors associated with the N balance method (Appendix A) which concluded that urethral catheterization increases the incidence of leukocytes, blood and nitrite in urine. It is very likely that this increase in leukocytes, blood and nitrites increased N excretion affecting the estimation of N retention, but more research is needed to quantify this effect on whole body N retention. From the exploratory experiment it was also concluded that older sows had a higher incidence of urinary nitrites in early gestation. This higher incidence of urinary nitrite by older parities during early gestation coincides with a greater N excretion, but causality could not be determined. At the time of writing this report the urine samples from the 38 females reported in Appendix A were collected but not analyzed, it is expected to perform an ANOVA (and post hoc test) that determines

whether the presence of nitrites in urine is related with changes in urine N excretion and allows more conclusions to be drawn.

There are also systematics errors related to the estimation of the SID Lys efficiency. These errors are generated by the limitations of the NRC (2012) gestating sow model of which some are discussed in Chapter 1. Despite these possible systematic errors, there are clear differences in kSID Lys WB Pd by parity and stage of gestation.

The NRC (2012) gestating sow model manipulated the kSID Lys WB Pd to match the model with the available empirical data. The manipulated kSID Lys WB Pd was estimated by the current authors based on the output of the NRC (2012) gestation sow model and was 0.42 for gilts, 0.41 for P1 sows and 0.40 for P2 sows throughout gestation. The results of the current study showed that for early gestation, gilts and P1 sows are more efficient at utilizing SID Lys than the NRC (2012) model expected. Thus, the SID Lys requirement for gilts and sows in early gestation may be overestimated, in fact when calculated kSID Lys WB Pd were included in the NRC (2012) gestating sow model, the SID Lys requirements decreased in early gestation from 10.47 to 6.19 g/d for gilts, 8.65 to 6.33 g/d for P1 sows and from 7.37 to 6.7 g/d for P2 sows; these results agrees with Navales et al. (2018) and suggest that the NRC (2012) gestating sow model is overestimating the SID Lys requirements in early gestation. When calculated kSID Lys WB Pd were included in the NRC (2012) gestating sow model for mid gestation the results did not agree with the empirical data; for mid gestation the SID Lys requirement increased from 9.7 to 10.7 g/d for gilts and from 7.9 to 8.5 g/d for P1 sows which do not agree with Navales et al. (2018) who reported that the NRC (2012) gestating sow model is overestimating the SID Lys requirements in mid gestation. When the kSID Lys WB Pd calculated for late gestation

were included in the NRC (2012) gestating sow model, the SID Lys requirement decrease from 16.2 to 13.3 g/d for gilts, 14 to 11.3 g/d for P1 sows and 12 to 7.4 g/ for P2 sows which does not agree with the empirical estimated SID Lys requirements (Dourmad and Etienne, 2002; Samuel, 2011; NRC, 2012; Navales et al., 2018).

Although the present study provides the model with more information about the kSID Lys WB Pd, the model does not seem to improve its predictive power for mid and late gestation. A possible explanation for this lack of model improvement is given that the AA requirements are the sum of those required for maintenance and protein retention divided by the kSID Lys WB Pd, the estimation of protein retention and maintenance may be misestimated. The model does not consider processes occurring during gestation such as AA biosynthesis which may cause an underestimation of the Lys retained. Lysine can be used to biosynthesize non-protein nitrogenous compounds as carnitine, and non-essential AA (Wu et al., 2010). A possible way to take into account (and try to reduce) the AA biosynthesis is to consider the dynamic AA ratio throughout gestation and based on the ideal protein concept provide the animal with diets that matches the AA profile necessary to deposit proteins at any given day of gestation.

Additionally, the reported differences in the kSID Lys WB Pd together with the exploratory experiment shown in Appendix A give insights on how to develop possible strategies for increasing the efficiency of N utilization. For example, because older sows have a greater incidence of urinary nitrites, and the main source of endogenous nitrate is the L-arginine-nitric oxide pathway, supplementation with arginine to older sows may help to reduce its biosynthesis from other AA and possibly increasing the efficiency of N utilization.

The results of the current research suggest the need for parity-segregated phase feeding of pregnant sows and provides significant information on how SID Lys requirement estimation can be improved. It is also suggested that the NRC (2012) gestating sow model be reviewed. Better estimation of AA requirements allows balanced diets to be formulated that reduce nutrient excretion, which is necessary to reduce the environmental impact of pig production and associated costs.

APPENDIX A

Urethral catheterization increases the incidence of leukocytes, blood and nitrites in urine.

Introduction

Nitrogen retention has been shown to be overestimated when determined by N balance and the difference is important (up to 16%) and variable when compared with the slaughter technique. (Just et al., 1982; Quiniou et al., 1995; Lenis et al., 1999; De Lange et al., 2001). The use of Foley catheters is the standard method to accomplish total urine collection during N-balance studies using pregnant sows (Van den Brand et al., 2000; Dourmad and Etienne, 2002; Srichana, 2006; Miller et al., 2016). Bacteriuria is associated with the use of catheters, especially when the collecting tube connected to the catheter has an open end (Warren, 2001); urethral inflammation is also associated with catheterization (Edwards and Trott, 1973). Bacteriuria and urethral inflammation promote the appearance of cells and metabolites such as erythrocytes, leukocytes, epithelial cells, a variety of cytokines and nitrites (Ringsrud, 2001; Janeway et al., 2005). It has been reported that up to 70–90% of plasma nitrite is produced endogenously in humans and rodents (Kleinbongard et al., 2003) and may occur similarly in pigs.

Thus, the production of cells such as leukocytes and erythrocytes, metabolites such as nitrites and the microorganisms themselves are sources of non-dietary N that can affect the estimation of N retention under the N balance method using urethral

catheterization. Thus, the hypothesis of the present experiment is that urethral catheterization promotes the incidence of leukocytes, blood and nitrites in urine.

Materials and Methods

The experiment was conducted at South Dakota State University Swine Education and Research Facility, Brookings, SD. A total of 38 females (PIC 1050; n = 15 gilts, n = 6 P1 sows, n = 7 P2 sows, n = 4 P3 sows, n = 6 P4 sows) in 2 blocks were used in the experiment. Females were kept in gestation stalls (61 cm x 1.98 m) from breeding to 110 d of gestation and were offered a common gestation diet (3300 kcal/kg ME and 0.46% SID Lys) at a feed allocation per day (i.e. 2.27 kg/d) to maintain a target body condition score of 3 when they were not under experimental diets. Experimental diets and allocation are described in Section 2.4.2 of the current study. In each block, three 12-d N-balance periods were conducted with urethral catheterization starting at d 48, 65 and 103 of gestation.

Urine samples were collected immediately after catheterization (day 0) and every 24 hours for the next three days. Urinary reagent strips (10 Parameter Urinalysis Reagent Strips by QTest) were used to determine the presence of leukocytes, blood and nitrites. The incidence of leukocytes, blood and nitrites was calculated as the number of positive observations divided by the total number of observations. Regression analysis under the PROC MIXED procedure of SAS was used to assess the relationship between leukocytes, blood and nitrites incidence versus the time the catheter remained inserted, with block as a random effect. One-way ANOVA under the PROC MIXED procedure of SAS was used to determine whether there were statistically significant differences between leukocytes,

blood and nitrites by parity or diet with block as a random effect. For all analyses, a $P < 0.05$ was considered significant.

Results and Discussion

For each N balance period there were positive linear relationships between leukocytes, blood and nitrites incidence vs versus the time the catheter remained inserted (Fig. Appx-1 A to C). Differences in leukocytes, blood and nitrites incidence by diet were not found for any N balance period. Differences in nitrites incidence by parity in N balance 1 were found ($P=0.0086$) as shown in Fig. Appx-1 D; the incidence of nitrites increased as parity increased to P3 sows and decreased for P4 sows. No other differences were detected.

There is clear evidence that the production of nitrates is an immune response. The main source of endogenous nitrate in mammals is the L-arginine-nitric oxide pathway. During systemic inflammatory reactions or infections, white blood cells and other cells express nitric oxide synthetase (iNOS), catalyzing the production of large amounts of nitric oxide from L-arginine (Lundberg et al., 2004). As up to 70–90% of plasma nitrite is produced endogenously, urine nitrite is also likely to come primarily from endogenous sources. An increase in N excretion from endogenous nitrites would reduce N retention values, which would also decrease kSID Lys WB Pd's estimated values.

It has been reported that the proinflammatory cytokine TNF-α increase iNOS expression (Chatterjee et al., 1999; Akama and Van Eldik, 2000). Ison et al. (2018) reported that multiparous sows have higher plasma concentrations of TNF-α than primiparous sows, therefore a greater iNOS expression; this would explain the increased

incidence of nitrites for older sows in early gestation. This increased incidence of urinary nitrite by older sows suggests that older sows have a more developed immune system compared to gilts. Besides, as older sows produce more nitrites than younger females, N retention may be further underestimated as parity increases.

In addition, the incidence of urinary nitrite seems to increase naturally as pregnancy progresses as demonstrated by the increase in nitrite incidence on day 0 with day of gestation. Urinary nitrite presence at day 0 was 11% for early gestation, 54% in mid gestation and 42% in late gestation; the last two being not different and greater than the urinary nitrite presence for early gestation. Furthermore, the data from day 0 suggests that the naturally occurring incidence of urinary nitrites (without the influence of catheterization) is also greater in older sows (gilts:23%; P1 sows:27%; P2 sows: 33%; P3 sows: 67%; P4 sows: 53%).

The data from the current study also suggest that there is a decline in the immune function of sows after the fourth pregnancy. It is widely recognized that many mammals experience a progressive age-related decline in immune function (Makinodan and Kay, 1980; Hirokawa, 1992), and that would explain the reduction in the urinary nitrite incidence difference from parity 3 to parity 4 sows in early gestation.

In conclusion, urethral catheterization increases the incidence of leukocytes, blood and nitrites in urine. More research is needed to determine the quantitative influence of the increase incidence of leukocytes, blood and nitrites caused by urethral catheterization in the estimation of N retention using the N balance method.

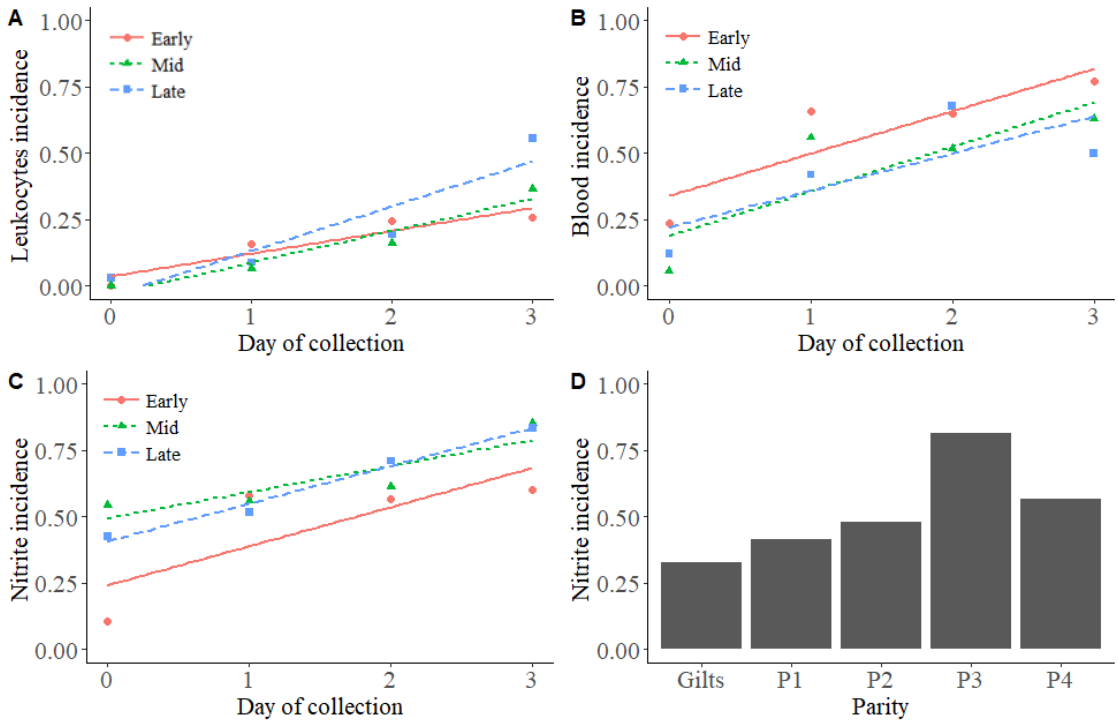

Figure Appx-1. The linear relationship between leukocytes (A), blood (B) and nitrites (C) incidence versus the day of collection where catheters remained inserted. Incidence was calculated as the number of positive observations divided by the total number of observations. Differences in nitrites incidence by parity in N balance 1 (D) were found (P=0.0086).

LITERATURE CITED

Akama, K. T., and L. J. Van Eldik. 2000. β-Amyloid stimulation of inducible nitric-oxide synthase in astrocytes is interleukin-1β-and tumor necrosis factor-α (TNFα)-dependent, and involves a TNFα receptor-associated factor-and NFκB-inducing kinase-dependent signaling mechanism. Journal of Biological Chemistry 275(11):7918-7924.

Balzani, A., H. Cordell, E. Sutcliffe, and S. Edwards. 2016. Sources of variation in udder morphology of sows. Journal of animal science 94(1):394-400.

Barbour, L. A., C. E. McCurdy, T. L. Hernandez, J. P. Kirwan, P. M. Catalano, and J. E. Friedman. 2007. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes care 30(Supplement 2):S112-S119.

Bérard, J., and G. Bee. 2010. Effects of dietary L-arginine supplementation to gilts during early gestation on foetal survival, growth and myofiber formation. Animal 4(10):1680-1687.

Chatterjee, P. K., G. M. Hawksworth, and J. S. McLay. 1999. Cytokine-stimulated nitric oxide production in the human renal proximal tubule and its modulation by natriuretic peptides: A novel immunomodulatory mechanism? Nephron Experimental Nephrology 7(5-6):438-448.

De Lange, C., A. Gillis, and G. Simpson. 2001. Influence of threonine intake on whole-body protein deposition and threonine utilization in growing pigs fed purified diets. Journal of animal science 79(12):3087-3095.

Dourmad, J.-Y., M. Etienne, A. Valancogne, S. Dubois, J. van Milgen, and J. Noblet. 2008. InraPorc: a model and decision support tool for the nutrition of sows. Animal Feed Science and Technology 143(1-4):372-386.

Dourmad, J., M. Etienne, J. Noblet, and D. Causeur. 1997. Prediction de la composition chimique des truies reproductrices a partir du poids vif et de l'epaisseur de lard dorsal. J. Rech. Porcine Fr 29:255-262.

Dourmad, J., J. Noblet, and M. Etienne. 1998. Effect of protein and lysine supply on performance, nitrogen balance, and body composition changes of sows during lactation. Journal of animal science 76(2):542-550.

Dourmad, J. Y., and M. Etienne. 2002. Dietary lysine and threonine requirements of the pregnant sow estimated by nitrogen balance. J Anim Sci 80(8):2144-2150.

Duée, P., A. Rérat, J. Jung, M. Serezat, and J. Lebost. 1975. Etude du besoin en lysine de la truie gestante nullipare. In: Annales de zootechnie. p 447-464.

Edwards, L., and P. A. Trott. 1973. Catheter-induced urethral inflammation. The Journal of urology 110(6):678-681.

Étienne, M., and J. Y. Dourmad. 2002. Dietary lysine and threonine requirements of the pregnant sow estimated by nitrogen balance1,2. Journal of Animal Science 80(8):2144-2150. doi: 10.1093/ansci/80.8.2144

Everts, H., and R. A. Dekker. 1995. Effect of protein supply during pregnancy on body composition of gilts and their products of conception. Livestock Production Science 43(1):27-36. doi: https://doi.org/10.1016/0301-6226(95)00004-5

Hirokawa, K. 1992. Understanding the mechanism of the age-related decline in immune function. NUTRITION REVIEWS-WASHINGTON- 50:361-361.

Ison, S. H., S. Jarvis, S. A. Hall, C. J. Ashworth, and K. M. Rutherford. 2018. Periparturient behavior and physiology: further insight into the farrowing process for primiparous and multiparous sows. Frontiers in veterinary science 5

Janeway, C. A., P. Travers, M. Walport, and M. Shlomchik. 2005. Immunobiology: the immune system in health and disease.

Jansson, T., V. Scholtbach, and T. L. Powell. 1998. Placental Transport of Leucine and Lysine Is Reduced in Intrauterine Growth Restriction1. Pediatric research 44(4):532.

Ji, F., W. Hurley, and S. Kim. 2006. Characterization of mammary gland development in pregnant gilts. Journal of animal science 84(3):579-587.

Just, A., J. Fernandez, and H. Jorgensen. 1982. Nitrogen balance studies and nitrogen retention, Digestive physiology in the pig No. 12. Institut national de la Recherche Agronomique Paris. p. 207-215.

Kim, S., I. Osaka, W. Hurley, and R. Easter. 1999. Mammary gland growth as influenced by litter size in lactating sows: impact on lysine requirement. Journal of Animal Science 77(12):3316-3321.

Kim, S. W., and G. Wu. 2009. Regulatory role for amino acids in mammary gland growth and milk synthesis. Amino acids 37(1):89-95.

Kim, S. W., G. Wu, and D. H. Baker. 2005. Ideal protein and dietary amino acid requirements for gestating and lactating sows. Pig News and Information 26(4):91N.

Kleinbongard, P., A. Dejam, T. Lauer, T. Rassaf, A. Schindler, O. Picker, T. Scheeren, A. Gödecke, J. Schrader, and R. Schulz. 2003. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. Free Radical Biology and Medicine 35(7):790-796.

Knight, J., F. W. Bazer, W. Thatcher, D. Franke, and H. Wallace. 1977. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. Journal of animal science 44(4):620-637.

Landgraf, S., A. Susenbeth, P. Knap, H. Looft, G. Plastow, E. Kalm, and R. Roehe. 2006. Allometric association between in vivo estimation of body composition during growth using deuterium dilution technique and chemical analysis of serial slaughtered pigs. Animal Science 82(2):223-231.

Lenis, N. P., H. T. van Diepen, P. Bikker, A. W. Jongbloed, and J. van der Meulen. 1999. Effect of the ratio between essential and nonessential amino acids in the diet on utilization of nitrogen and amino acids by growing pigs. Journal of animal science 77(7):1777-1787.

Levesque, C. L., S. Moehn, P. B. Pencharz, and R. O. Ball. 2011. The threonine requirement of sows increases in late gestation. J Anim Sci 89(1):93-102. doi: 10.2527/jas.2010-2823

Lewis, R., S. Brooks, I. Crocker, J. Glazier, M. Hanson, E. Johnstone, N. Panitchob, C. Please, C. Sibley, and K. Widdows. 2013. Modelling placental amino acid transfer− From transporters to placental function. Placenta 34:S46-S51.

Llames, C. R. 1994. Determination of amino acids in feeds: collaborative study. Journal of Association of Official Analytical Chemists 77:1362-1402.

Lundberg, J. O., E. Weitzberg, J. A. Cole, and N. Benjamin. 2004. Nitrate, bacteria and human health. Nature Reviews Microbiology 2(7):593.

Makinodan, T., and M. M. Kay. 1980. Age influence on the immune system, Advances in immunology No. 29. Elsevier. p. 287-330.

Miller, E., L. Huber, C. Levesque, and C. F. de Lange. 2018. Variability in daily urinary nitrogen excretion of gestating gilts does not affect estimates of nitrogen retention. Canadian Journal of Animal Science 98(3):603-605.

Miller, E., C. Levesque, N. Trottier, and C. de Lange. 2016. Dynamics of nitrogen retention in gestating gilts at two feeding levels. Journal of animal science 94(8):3353-3361.

Moe, P., H. Tyrrell, and W. Flatt. 1971. Energetics of body tissue mobilization. Journal of Dairy Science 54(4):548-553.

Mok, C. 2015. Using the indicator amino acid oxidation technique to study threonine requirements in horses fed different feed compositions.

Navales, R., B. Thaler, and C. Levesque. 2018. Efficiency of Utilizing Standardized Ileal Digestible Lys for Whole Body Protein Retention in Pregnant Gilts during Early, Mid and Late Gestation. Journal of Animal Science 96(suppl_2):67-67.

Noblet, J., W. Close, R. Heavens, and D. Brown. 1985. Studies on the energy metabolism of the pregnant sow: 1. Uterus and mammary tissue development. British Journal of Nutrition 53(2):251-265.

NRC. 1998. Nutrient Requirements of Swine: 10th Revised Edition. The National Academies Press, Washington, DC.

NRC. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. The National Academies Press, Washington, DC.

Père, M.-C., and M. Etienne. 2000. Uterine blood flow in sows: effects of pregnancy stage and litter size. Reproduction Nutrition Development 40(4):369-382.

Pomar, C., I. Kyriazakis, G. Emmans, and P. Knap. 2003. Modeling stochasticity: Dealing with populations rather than individual pigs. Journal of Animal Science 81(14_suppl_2):E178-E186.

Potthoff, R. F. 1966. Statistical aspects of problem of biases in psychological tests, North Carolina State University. Dept. of Statistics.

Quiniou, N., S. Dubois, and J. Noblet. 1995. Effect of dietary crude protein level on protein and energy balances in growing pigs: comparison of two measurement methods. Livestock Production Science 41(1):51-61.

Ringsrud, K. M. 2001. Cells in the urine sediment. Laboratory Medicine 32(3):153-155.

Rippel, R., B. Harmon, A. Jensen, H. Norton, and D. Becker. 1965. Essential amino acid supplementation of intact proteins fed to the gravid gilt. Journal of animal science 24(2):373-377.

Rozeboom, D., J. Pettigrew, R. Moser, S. Cornelius, and S. El Kandelgy. 1994. In vivo estimation of body composition of mature gilts using live weight, backfat thickness, and deuterium oxide. Journal of animal science 72(2):355-366.

Samuel, R. 2011. Simultaneous measurement of protein and energy metabolism and application to determine lysine requirements in sows.

Samuel, R. S. 2008. Maintenance energy metabolism in non-pregnant and pregnant sows, University of Alberta.

Sohail, M. A., D. J. Cole, and D. Lewis. 1978. Amino acid requirements of the breeding sow: the dietary lysine requirement during pregnancy. Br J Nutr 39(3):463-468.

Srichana, P. 2006. Amino acid nutrition in gestating and lactating sows. ProQuest.

Taylor, J. 1997. Introduction to error analysis, the study of uncertainties in physical measurements.

Thomas, L. L., R. D. Goodband, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, and S. S. Dritz. 2018. Effect of parity and stage of gestation on growth and feed efficiency of gestating sows. Journal of animal science 96(10):4327-4338.

Van den Brand, H., M. Heetkamp, N. Soede, J. Schrama, and B. Kemp. 2000. Energy balance of lactating primiparous sows as affected by feeding level and dietary energy source. Journal of animal science 78(6):1520-1528.

van Milgen, J., and J.-Y. Dourmad. 2015. Concept and application of ideal protein for pigs. Journal of Animal Science and Biotechnology 6(1):15.

Warren, J. W. 2001. Catheter-associated urinary tract infections. International journal of antimicrobial agents 17(4):299-303.

Weaver, B., and K. L. Wuensch. 2013. SPSS and SAS programs for comparing Pearson correlations and OLS regression coefficients. Behavior Research Methods 45(3):880-895. (journal article) doi: 10.3758/s13428-012-0289-7

Woerman, R. L., and V. Speer. 1976. Lysine requirement for reproduction in swine. Journal of animal science 42(1):114-120.

Wu, G., F. Bazer, R. Burghardt, G. Johnson, S. Kim, X. Li, M. Satterfield, and T. Spencer. 2010. Impacts of amino acid nutrition on pregnancy outcome in pigs: mechanisms and implications for swine production. Journal of animal science 88(suppl_13):E195-E204.

Wu, G., F. W. Bazer, J. Hu, G. A. Johnson, and T. E. Spencer. 2005. Polyamine synthesis from proline in the developing porcine placenta. Biology of reproduction 72(4):842-850.

Wu, G., T. L. Ott, D. A. Knabe, and F. W. Bazer. 1999. Amino acid composition of the fetal pig. The Journal of nutrition 129(5):1031-1038.

Zervas, S., and R. T. Zijlstra. 2002. Effects of dietary protein and fermentable fiber on nitrogen excretion patterns and plasma urea in grower pigs. J Anim Sci 80(12):3247-3256.

Zhang, S., F. Chen, Y. Zhang, Y. Lv, J. Heng, T. Min, L. Li, and W. Guan. 2018. Recent progress of porcine milk components and mammary gland function. Journal of animal science and biotechnology 9(1):77.